SUPPORT

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UNION CARBIDE CORPORATION
P. O. Box 670, Bound Brook, NJ 08805-0670

CERTIFIED MAIL
RETURN RECEIPT REQUESTED

January 16, 2001

MR 43331

TSCA Document Processing Center (7407)
Office of Pollution Prevention & Toxics
U.S. Environmental Protection Agency
401 M Street, SW
Washington, DC 20460

8EHQ-98-14257

Attention: 8(e) Coordinator

RE: 17-Oxabicyclo [4.1.0] heptane, 3-ethenyl- [VCMX; CASRN 106-86-5]
8EHQ-98-14257

Dear Sir or Madam:

Union Carbide Corporation ("Union Carbide") herewith submits the final report as described below as a supplement to its August 31, 1998 TSCA 8(e) submission - [8EHQ-98-14257].

Vinyl Cyclohexene Monoxide (VCMX): A 13-Week Inhalation Toxicity Study in Mice and Rats Via Whole-Body Exposures 12-Crown-4: A 4-Week Inhalation Toxicity Study in Rats Via Whole-Body Exposures; Report Number 97U1660, October 20, 2000; Huntingdon Life Sciences.

Contain NO CBI

89010000092

Very truly yours,

Imogene E. Treble

Imogene E. Treble, Ph.D.
Assistant Director
Chemical Control Compliance

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Attachments

Huntingdon
Life Sciences

OECD TEST GUIDELINE NO. 413
EPA/TSCA TEST GUIDELINE NO. 798.2450

STUDY NO. 97-6117
SPONSOR STUDY NO. 97U1660

CHEMICAL NAME: 1,2-EPOXY-4-VINYLCYCLOHEXANE

VINYL CYCLOHEXENE MONOXIDE (VCMX): A 13-WEEK INHALATION TOXICITY STUDY IN MICE AND RATS VIA WHOLE-BODY EXPOSURES

Amended Final Report

Volume I of IV

Submitted to: Union Carbide Corporation
39 Old Ridgebury Road
Danbury, CT 06817-0001

Final Report Date: 26 May 2000
Amended Report Date: 20 October 2000

STATEMENT OF CONFIDENTIALITY CLAIMS

Union Carbide Corporation Business Confidential:

Not to be released outside Union Carbide Corporation without the written consent of the Union Carbide Corporation.

GLP COMPLIANCE

This study was conducted in compliance with the Organization for Economic Cooperation and Development (OECD) Principles of Good Laboratory Practices (ENV/MC/CHEM[98]17) and EPA Good Laboratory Practices – TSCA (40 CFR 792) with the following exceptions:

> The Testing Facility lacks knowledge of procedures used for feed and water analysis.
>
> The Sponsor did not provide an expiration date for the test material.
>
> In accordance with 40 CFR 792.35(b)(3) a study-specific inspection was not conducted for the processing of tissues, blocks or slides, or for the pathological evaluations conducted at the HLS-ERC test site. However, process-based inspections were conducted at that test site in accordance with the OECD principles of GLP.

These exceptions were not considered to affect the interpretation or results of the study.

Gary M. Hoffman, B.A., DABT
Study Director

20 Oct 00
Date

SIGNATURE PAGE

SCIENTIST

The following Scientist was responsible for the overall conduct of this study. Departmental supervisory personnel are listed on the personnel page of this report (Appendix GG).

Gary M. Hoffman, B.A., DABT
Study Director

26 May 00
Date

SCIENTIFIC REVIEW

The following Scientist has reviewed and approved this report.

Sylvie J. Gosselin, D.V.M., Ph.D., Diplomate A.C.V.P.
Vice President, Research

24 May 2000
Date

QUALITY ASSURANCE STATEMENT[a]

Listed below are the dates that this study was inspected by the Quality Assurance Unit of Huntingdon Life Sciences, East Millstone, New Jersey, and the dates that findings were reported to the Study Director and Management.

Type of Inspection	Date(s) of Inspection	Reported to Study Director and Management
GLP Protocol Review	9 Dec 97	9 Dec 97
Exposure and Monitoring	27 Jan 98	27 Jan 98
Body Weights and Feeder Weights	6 Feb 98	6 Feb 98
Physical Observations	4 Mar 98	5 Mar 98
Terminal Blood Collection and Sacrifice (Mice)	29 Apr 98	29 Apr 98
Urine Collection and Analysis	30 Apr 98	30 Apr 98
Terminal Ophthalmology	28 May 98	28 May 98
Recovery Sacrifice (Rats)	29 May 98	29 May 98
In-Life Report and Raw Data Review	24 Sep 98 and 28 Sep 98 to 1 Oct 98	1 Oct 98
Sponsor Comments	17 and 18 Oct 00	18 Oct 00

N S Iacono

Nicki S. Iacono
Manager, Quality Assurance

20 Oct 00
Date

[a]The Quality Assurance Statement originally signed on 04 November 1999 was re-signed due to Sponsor requested revisions to the final report.

QUALITY ASSURANCE STATEMENT

VINYL CYCLOHEXENE MONOXIDE (VCMX)

A 13-WEEK INHALATION TOXICITY STUDY IN MICE AND RATS
VIA WHOLE-BODY EXPOSURES

The following inspections and audits have been carried out in relation to the histopathology phase of the study

Study Phase	Date of Inspection	Date of Reporting
Process Based Inspections		
Tissue Trimming	25 August 1998	25 August 1998
Embedding	24 June 1998	26 June 1998
Decalcification	22 September 1998	22 September 1998
Sectioning	15 July 1998	15 July 1998
Staining	19 August 1998	24 August 1998
Slide Collation	19 May 1998	20 May 1998
Light Microscopy and Data Entry	9 June 1998	9 June 1998
Report Audit	10 May 2000	10 May 2000

Process based inspections: At or about the time this phase of the study was in progress inspections of routine and repetitive procedures employed on this type of study were carried out. These were conducted and reported to appropriate Company Management as indicated above

Report Audit: This report has been audited by the Quality Assurance Department. This audit was conducted and reported to the Principal Investigator and Company Management as indicated above.

The methods, procedures and observations were found to be accurately described and the reported results of this phase of the study to reflect the raw data.

SJWatts .... 15 MAY 2000 ....

S. J. Watts, C.Biol., M.I.Biol.,
Principal Auditor
Department of Quality Assurance
Huntingdon Life Sciences Ltd.

Date

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SUMMARY

This study, conducted for Union Carbide Corporation, was designed to assess the toxic effects of Vinyl Cyclohexene Monoxide (VCMX), CAS No. 106-86-5, when administered by whole-body inhalation as a vapor to Sprague-Dawley CD® rats and CD-1® mice (10 animals/sex/species for the low and mid exposure levels; 20 animals/sex/species for the air control and high exposure levels) for 6 hours per day, 5 days per week, for at least 13 weeks at target concentrations of 0 (air control), 20, 80 and 200 (mice) or 400 (rats) parts per million (ppm) in air. Following the treatment period, half of the animals from the air control and high exposure levels were held for a 4-week recovery period. Exposure levels were determined by infrared spectrophotometric (MIRAN®) analysis at least 4 times per chamber per day. Particle size distribution measurements were made once per chamber per day using a TSI Aerodynamic Particle Sizer.

Physical observations, ophthalmoscopic examinations, body weight, food consumption measurements, hematology, clinical chemistry and urinalyses were performed on all animals at selected intervals during the treatment and recovery periods.

After at least 13 weeks of treatment or a 4-week recovery period, all survivors were sacrificed, selected organs were weighed and organ/body weight and organ/brain weight ratios calculated. Complete macroscopic postmortem examinations and histopathological evaluation of selected tissues were conducted on all animals.

The mean (± standard deviation) analytical exposure concentrations were determined to be 0.0±0.0, 19.9±1.0, 78.9±4.6, 196.3±8.3 (mice) and 395.5±17.6 (rats) ppm for the air control, low, mid and high exposure groups, respectively. Particle size distribution determinations indicated, as expected, that the test atmospheres were vapor only.

Three female mice from the 200 ppm exposure group were found dead on test days 39, 56 and 74 days, respectively. No cause of death was determined but these deaths were considered treatment-related. All other test animals survived the exposures and recovery periods.

Observations of the test animals were generally unremarkable while in the exposure chambers. During non-exposure periods, the 400 ppm exposed rats were noted with nasal discharge and facial staining both of which abated during the recovery period. During non-exposure periods, the 200 ppm exposed mice were noted with yellow ano-genital staining during both the exposure and recovery periods. Ophthalmology examinations did not reveal any test material related abnormalities in either species.

SUMMARY (CONT.)

The high exposure male rats and mice had decreased body weights during the exposure period with a recovery after termination of exposures. Food consumption measurements and hematology/clinical chemistry measurements were unremarkable. Urinalysis indicated decreased pH values in 400 ppm exposed rats after 13 weeks of exposure and increased ketone levels in these same animals at both sacrifice intervals.

Organ weight measurements were generally unremarkable with the exception of significantly decreased ovary weights in 200 ppm exposed mice at both sacrifice intervals. Macroscopic postmortem evaluations were unremarkable. Microscopic postmortem evaluations revealed degeneration of the nasal epithelium in rats and mice at all exposure levels of VCMX, without recovery. Microscopic postmortem evaluations also revealed degeneration (reduced number of follicles) of the ovaries in the 200 ppm exposed mice, without recovery.

In conclusion, inhalation exposure of rats and mice to VCMX at concentrations of 20 ppm and higher, for 13 weeks, resulted in an exposure-level-related increase in nasal epithelium changes in both species, which were not completely reversible in high-concentration animals (mice, 200ppm; rats, 400 ppm) held for a 4-week recovery period. These nasal effects were seen without any corresponding lung changes indicating a localized effect of test material exposure within the respiratory tract. Based on these findings, a NOEL for inhalation exposure to Vinyl Cyclohexene Monoxide (VCMX) was not established in this study. Systemic effects were limited to mortality and non-reversible ovarian degeneration in female mice exposed to the 200 ppm concentration, decreased body weight gains in male rats and mice, decreased urine pH and other minor urinary changes at the highest concentration (400 and 200 ppm, respectively).

1. INTRODUCTION

This study was conducted for Union Carbide Corporation, using Sprague-Dawley CD® rats and CD-1® mice to assess the potential inhalation toxicity of Vinyl Cyclohexene Monoxide (VCMX) administered via whole-body exposures for at least 13 weeks followed by a 4-week recovery period. The test material was generated as a vapor at target concentrations of 20, 80, 200 (mice only) and 400 (rats only) parts per million (ppm). Species of test animal, method and route of test material administration and exposure levels were determined by the Sponsor.

2. MATERIALS AND METHODS

2.1. REGULATORY REFERENCES

2.1.1. TEST GUIDELINES

Health Effects Test Guidelines; Office of Pesticides and Toxic Substances; United States Environmental Protection Agency (EPA/TSCA), Subchronic Exposure Inhalation Toxicity, section 798.2450; published September 27, 1985 and amended on May 16, 1989.

The Organization for Economic Cooperation and Development (OECD) Test Guidelines No. 413 "Subchronic Inhalation Toxicity" Adopted 12 May 1981.

2.1.2. GOOD LABORATORY PRACTICES

This study was conducted in compliance with OECD Principles of Good Laboratory Practices (ENV/MC/CHEM[98]17) and Part 792 of 40 CFR (EPA Good Laboratory Practices - TSCA).

2.1.3. ANIMAL WELFARE ACT COMPLIANCE

This study complied with all appropriate parts of the Animal Welfare Act regulations: 9 CFR Parts 1 and 2 Final Rules, Federal Register, Volume 54, No. 168, August 31, 1989, pp. 36112-36163 effective October 30, 1989 and 9 CFR Part 3 Animal Welfare Standards; Final Rule, Federal Register, Volume 56, No. 32, February 15, 1991, pp. 6426-6505 effective March 18, 1991.

2.1.4. FACILITIES MANAGEMENT/ANIMAL HUSBANDRY

Currently acceptable practices of good animal husbandry were followed e.g., *Guide for the Care and Use of Laboratory Animals*; National Academy Press, 1996. Huntingdon Life Sciences, East Millstone, New Jersey is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC).

2.2. STUDY MANAGEMENT

2.2.1. SPONSOR

Union Carbide Corporation
39 Old Ridgebury Road
Danbury CT 06817-0001

2.2.2. SPONSOR REPRESENTATIVE

John P. Van Miller, Ph.D., DABT

2.2.3. TESTING FACILITY

Huntingdon Life Sciences
Princeton Research Center
P.O. Box 2360
Mettlers Road
East Millstone, New Jersey 08875-2360

2.2.4. STUDY DIRECTOR

Gary M. Hoffman, B.A., DABT

2.3. EXPERIMENTAL DESIGN

The following tables are the experimental design of the study. The actual numbers of animals in each group might vary as animals died.

Rats

Group	Exposure Level[a]	Number of Animals									
		Initial		Clinical Laboratory Studies[b]/Necropsy				Microscopic Pathology[c]			
				Termination		Recovery		Termination		Recovery	
	(ppm)	M	F	M	F	M	F	M	F	M	F
IB	0	20	20	10	10	10	10	10	10	10	10
II	20	10	10	10	10	0	0	10	10	0	0
III	80	10	10	10	10	0	0	10	10	0	0
IVB	400	20	20	10	10	10	10	10	10	10	10

[a]Exposure levels were selected by the Sponsor on the basis of available toxicity data (see Section 2.14.3).
[b]Only urinalysis was performed during the recovery interval.
[c]Microscopic evaluations of selected tissues were performed for Groups I and IV at termination. Evaluations of selected tissues and target tissues were performed for Groups II and III at termination. Evaluations of target tissues were performed at recovery.

M = males, F = females

Mice

Group	Exposure Level[a]	Number of Animals									
		Initial		Clinical Laboratory Studies[b]/Necropsy				Microscopic Pathology[c]			
				Termination		Recovery		Termination		Recovery	
	(ppm)	M	F	M	F	M	F	M	F	M	F
IA	0	20	20	10	10	10	10	10	10	10	10
II	20	10	10	10	10	0	0	10	10	0	0
III	80	10	10	10	10	0	0	10	10	0	0
IVA	200	20	20	10	10	10	10	10	10	10	10

[a]Exposure levels were selected by the Sponsor on the basis of available toxicity data (see Section 2.14.3).
[b]Clinical pathology parameters were not performed on animals sacrificed at recovery.
[c]Microscopic evaluations of selected tissues were performed for Groups I and IV at termination. Evaluations of selected tissues and target tissues were performed for Groups II and III at termination. Evaluations of target tissues were performed at recovery.

M = males, F = females

2.4. STUDY DATES

2.4.1. STUDY INITIATION

22 December 1997 (Date Study Director signed the Protocol)

2.4.2. DATE OF ANIMAL RECEIPT

13 January 1998

2.4.3. INITIATION OF EXPOSURES

27 January 1998 (Experimental Start Date)

2.4.4. TERMINATION OF EXPOSURES

Rats
30 April 1998

Mice
28 April 1998

2.4.5. TERMINAL SACRIFICE

Rats
30 April 1998 and 1 May 1998

Mice
29 April 1998

2.4.6. RECOVERY SACRIFICE

Rats
29 May 1998

Mice
28 May 1998

2.4.7. STUDY TERMINATION

26 May 2000 (Date Final Report is signed by the Study Director)

2.5. TEST MATERIAL

Vinyl Cyclohexene Monoxide
Chemical name: 1,2-Epoxy-4-vinylcyclo-hexane

2.5.1. CAS NUMBER

106-86-5

2.5.2. CAS NAME

7-Oxabicyclo {4.1.0}heptane, 3-ethenyl

2.5.3. MANUFACTURER

Union Carbide Corporation
Taft Plant
River Road, Route 18
P.O. Box 50
Hahnville, Louisiana 70057

2.5.4. SUPPLIER

Union Carbide Corporation
Chemical Marketing Concepts
200 Pickett District Road
New Milford, CT 06776

2.5.5. LOT NUMBER/BATCH NUMBER

TF-3-24551

2.5.6. PURITY

99.4% per COA (see Appendix DD)
99.1% (prestudy) and 99.0% (poststudy) per SCCR (see Appendix DD)

2.5.7. DENSITY

0.9598 g/mL per MSDS

2.5.8. DESCRIPTION

Slightly viscous translucent liquid

2.5.8. DESCRIPTION

Slightly viscous translucent liquid

2.5.9. DATE RECEIVED

10 December 1997

2.5.10. EXPIRATION DATE

Not available

2.5.11. PHYSICAL PROPERTIES

The nature of the test material and its solubility, melting/boiling point, vapor pressure and flammability are the responsibility of the Sponsor.

2.5.12. STORAGE

Room Temperature under Nitrogen

2.5.13. ARCHIVAL SAMPLE

A sample of test material is stored in the Archives of the Testing Facility.

2.5.14. ADDITIONAL SAMPLING

A sample of test material was shipped to the Sponsor laboratory in Charleston, WV before and after the study to determine the purity and stability.

2.5.15. DISPOSITION

Unused portion of the test material will be returned to the Sponsor at the completion of this study. Empty containers will be disposed of at the end of the study, upon authorization of the study Sponsor.

2.6. TEST ANIMALS

Albino Rats (Outbred) VAF/Plus®
Albino Mice (Outbred)

2.6.1. SPECIES

Albino Rats (Outbred) VAF/Plus®
Crl:CD® (SD) IGS BR

Albino Mice
CD-1® [Crl:CD-1®(ICR)BR]

2.6.2. SUPPLIER

Rats
Charles River Laboratories
Kingston, New York 12484

Mice
Charles River Laboratories
Portage, Michigan 49081

2.6.3. JUSTIFICATION FOR ANIMAL SELECTION

The mouse and rat are rodent animal models commonly utilized in toxicity studies as recommended in the referenced guidelines. In addition, a historical data base is available for comparative evaluation.

2.6.4. NUMBER OF ANIMALS

Rats
Received
150 total (75 males, 75 females)

Placed On-test
120 total (60 males, 60 females)

Mice
Received
162 total (81 males, 81 females)

Placed On-test
120 total (60 males, 60 females)

Females were nulliparous and non-pregnant.

2.6.5. AGE AT RECEIPT (approximately)

4 weeks (rats and mice)

2.6.6. AGE AT INITIATION OF EXPOSURE (approximately)

6 weeks (rats and mice)

2.6.7. WEIGHT AT INITIATION OF EXPOSURE (GRAMS)

Rats

	Mean	**Range**
Male:	213	198 - 233
Female:	141	128 - 155

Mice

	Mean	**Range**
Male:	27	25 - 29
Female:	21	20 - 23

Individual weights of animals placed on test were within ±20% of the mean weight for each sex and species.

2.6.8. ACCLIMATION PERIOD

Animals were acclimated for 2 weeks. All animals were examined during the acclimation period to confirm suitability for study.

2.7. SELECTION

More animals than required for the study were purchased and acclimated. Animals considered unsuitable for the study on the basis of pretest physical examinations, outlying body weight data, or ophthalmoscopic examinations were eliminated prior to random selection for group assignment. Disposition of all animals not utilized in the study is maintained in the study file.

2.8. GROUP ASSIGNMENT

Animals considered suitable for study were distributed into 2 groups of 20 animals per sex per species in Groups I and IV and 2 groups of 10 animals per sex per species in Groups II and III. The animals were distributed by a computerized random sort program so that body weight means for each group were comparable.

2.9. ANIMAL IDENTIFICATION

Each rat was identified with a metal ear tag bearing its assigned animal number. The assigned animal number plus the study number comprised the unique animal number for each animal. If the tag was lost, it was replaced. In addition, each non-exposure cage was provided with a cage card which was color-coded for exposure level identification and contained study number and animal number information.

Each mouse was identified with a tail tattoo bearing its assigned animal number. The assigned animal number plus the study number comprised the unique animal number for each animal. In addition, each non-exposure cage was provided with a cage card which was color-coded for exposure level identification and contained animal number information.

2.10. VETERINARY CARE

Animals were monitored by the technical staff for any conditions requiring possible veterinary care and treated as necessary.

2.11. HUSBANDRY DURING NON-EXPOSURE PERIODS

2.11.1. HOUSING

Animals were doubly housed in elevated, stainless steel, wire mesh cages during the first week of the acclimation period and individually housed during the remainder of the acclimation period and all other non-exposure periods. The rats and mice were housed in separate rooms at receipt and for the entire study.

2.11.2. FOOD

Certified Rodent Diet, No. 5002; (Meal) (PMI Nutrition International, St. Louis, Missouri) was available without restriction. Fresh food was presented weekly for the rats and twice weekly for the mice.

2.11.3. ANALYSIS OF FEED

Analysis of each feed lot used during this study was performed by the manufacturer. Results are maintained on file at the Testing Facility.

2.11.4. WATER

Water was available without restriction via an automated watering system (Elizabethtown Water Company).

2.11.5. WATER ANALYSIS

Water analyses are conducted by Elizabethtown Water Company, Westfield, New Jersey (Raritan-East Millstone Plant) to ensure that water meets standards specified under the EPA Federal Safe Drinking Water Act Regulations (40 CFR Part 141). In addition, water samples are collected biannually from representative rooms in the Testing Facility; chemical and microbiological water analyses are conducted on these samples by a subcontract laboratory. Results of all water analyses are maintained on file at the Testing Facility.

2.11.6. CONTAMINANTS

There were no known contaminants in the feed or water which were expected to interfere with the results of this study.

2.11.7. ENVIRONMENTAL CONDITIONS

Light/Dark Cycle

Twelve hour light/dark cycle controlled via an automatic timer.

Temperature

Temperature was monitored and recorded twice daily and maintained to the maximum extent possible within the desired range. Excursions outside the specified range did not affect the integrity of the study.

Desired:	18 to 26°C
Actual:	20 to 27°C

Mice and rats were held in separate rooms. The range presented is the overall range for the two rooms.

Relative Humidity

Relative humidity was monitored and recorded once daily and maintained to the maximum extent possible within the desired range. Excursions outside the specified range did not affect the integrity of the study.

Desired:	30 to 70%
Actual:	26 to 72%

Mice and rats were held in separate rooms. The range presented is the overall range for the two rooms.

2.12. HUSBANDRY DURING EXPOSURE PERIODS

2.12.1. HOUSING

Animals were individually housed in stainless steel, wire mesh cages within 1000 liter stainless steel and glass whole-body exposure chambers. The placement of the animal in the whole-body exposure cage was rotated at each exposure to ensure uniform exposure of the animals. A description of the animal rotation is included in the raw data. Mice and rats were placed in separate chambers for Groups I and IV but in the same chambers for Groups II and III.

2.12.2. FOOD

None was provided during exposure.

2.12.3. WATER

None was provided during exposure.

2.12.4. ENVIRONMENTAL CONDITIONS

Chamber temperature and relative humidity were recorded every half-hour during exposure and maintained, to the maximum extent possible, within the ranges presented below. Excursions outside the specified range did not affect the integrity of the study.

Temperature

Desired:	20 to 24°C
Actual:	19 to 31°C

Relative Humidity

Desired:	40 to 60%
Actual:	16 to 80%

2.13. TEST MATERIAL PREPARATION

The test material was used as received.

2.14. TEST MATERIAL ADMINISTRATION AND CHAMBER OPERATION

2.14.1. ROUTE OF ADMINISTRATION

Inhalation, administered into the breathing zone of the animals as a vapor.

2.14.2. ROUTE JUSTIFICATION

The inhalation route is one of the potential routes of human exposure to this test material and is the route specified in the reference guidlelines.

2.14.3. EXPOSURE LEVELS

Group IA - 0 ppm (mice)
Group IB - 0 ppm (rats)
Group II - 20 ppm
Group III - 80 ppm
Group IVA - 200 ppm (mice)
Group IV - 400 ppm (rats)

These exposure levels were based on the results of a 9-day inhalation toxicity study (97-6112). This study showed that the rats tolerated the 400 ppm exposure level. However, this study showed mortality in the mice at the 400 ppm exposure level but not at the 200 ppm exposure level. Therefore, the 200 ppm exposure level was selected for the high level in mice for this study with lower levels of 80 and 20 ppm for both the mice and rats.

2.14.4. FREQUENCY OF EXPOSURE

Once daily, 6 hours/day

2.14.5. DURATION

The test material was administered for generally 5 days per week for at least 13 weeks for at least 66 exposures. Test material administration was continued through the day prior to necropsy.

2.14.6. PRE-STUDY TRIALS

Trials were performed to evaluate the optimal set of conditions and equipment to generate a stable atmosphere at the targeted exposure levels. During this time, samples were taken to determine the distribution of the test material in the exposure chamber.

2.14.7. CHAMBER OPERATION

Each whole-body exposure chamber had a volume of approximately 1000 Liters. The exposure chambers were operated dynamically under slight negative pressure. The chamber airflow rate, time for air change and 99% equilibrium time (T99) for each group are summarized on the next page:

Group	Airflow	Air Change	T99
	(Lpm)	(min)	(min)
IA (mice)	207	4.8	22
IB (rats)	204	4.9	23
II	201	5.0	23
III	201	5.0	23
IVA (mice)	218	4.6	21
IVB (rats)	201	5.0	23

This chamber size and airflow rate was considered adequate to maintain the animal loading factor below 5% and oxygen above 19%. The chamber was exhausted through a system consisting of a coarse filter, a HEPA filter and activated charcoal bed.

All animals remained in the chamber for a minimum of 30 minutes at the end of the exposure. During this time the chamber was operated at approximately the same flow rate as used during the exposure, using clean air only.

Recordings of airflow rate and static pressure were made every half-hour during exposure.

Refer to Figure 1 and Appendix BB for equipment details.

2.14.8. EXPOSURE PROCEDURE

Groups IA and IB

House line nitrogen was delivered, at a flowrate of 207 and 204 Lpm for Groups IA and IB, respectively, from a regulator and backpressure gauge via ¼" Teflon tubing through a flowmeter, regulated by a metering valve, into the turret of the exposure chamber.

Groups II, III, IVA and IVB

For Groups II and III, the test material was delivered from a 10 cc syringe and syringe pump at the initial setting of 1.3 mL/hr and 60 cc syringe and syringe pump at the initial setting of 5.3 mL/hr, respectively. For Groups IVA and IVB, the test material was delivered from an Erlenmeyer flask to a fluid metering pump at the initial pump settings of 58% and 31%, respectively. For all groups, the test material flowed from the pumps, via

1/8" Teflon® tubing onto a heated glass helix of a counter current volatilization chamber. As the test material flowed down the helix, it was vaporized by a generating gas consisting of house line nitrogen and air. The house line air was delivered from a regulator and backpressure gauge via ¼" Teflon® tubing through a flowmeter, regulated by a metering valve, to a Swagelok® "T" where it combined with the nitrogen flow. The house line nitrogen was delivered from a regulator and backpressure gauge via ¼" Teflon® tubing through a flowmeter, regulated by a metering valve, to a Swagelok® "T" where it combined with the air flow. The combined nitrogen/air flowed via ¼" metal tubing through a furnace (used to heat the generating gas) and then entered the bottom of the volatilization chamber via a ground glass ball and socket joint, and flowed up through the chamber volatilizing the test material. The test material laden gas flowed through ¾" tubing at the top of the volatilization chamber into the turret of the exposure chamber, where it mixed with room air as it was drawn into the exposure chamber. The heat to the glass helix was controlled by a variable autotransformer. The furnace temperature was controlled by an autotransformer. These autotransformers operated by regulating the voltage being delivered to the heat sources. A digital alarm module was used to monitor the temperature within the volatilization chamber, which was maintained between 115°F - 120°F. The alarm on the module was set to a temperature of 122°F to warn when the test material flash point (126°F/52°C) was being approached.

Refer to Figure 1 and Appendix BB for equipment details.

2.15. EXPOSURE CHAMBER SAMPLING

2.15.1. CHAMBER SAMPLING

Determination of the Vinyl Cyclohexene Monoxide (VCMX) exposure levels were made using a MIRAN® Ambient Air analyzer (infrared spectrophotometer) equipped with a strip chart recorder. The test atmosphere was drawn through the MIRAN® and measurements were recorded at least four times during the exposures from the normal sampling portal. The exposure levels were determined by comparison of the measured absorbance to a calibrated response curve constructed using the same instrument settings.

Refer to Appendix BB for equipment details.

2.15.2. PARTICLE SIZE DISTRIBUTION ANALYSIS

During each exposure, particle size distribution samples were taken using a TSI Aerodynamic Particle Sizer. A computer was used to program the system to the settings prior to sampling. A printer was used to record the information. Samples were pulled once daily for 20 seconds at the flow rate of 5 Lpm. The samples were drawn from the normal sampling port of the chamber using a stopper and a ½" tubing connected to a ½" stainless steel tubing, which was extended from the chamber to the particle sizer. The particle size distributions were calculated by the computer and printed out based on the amount of material collected by the particle sizer.

Refer to Appendix BB for equipment details.

2.15.3. NOMINAL EXPOSURE CONCENTRATION

The nominal exposure concentrations (ppm) were determined by weighing the generation apparatus containing the test material before and after the exposure and calculated as follows:

$$C(\text{ppm}) = \frac{\text{weight change (g)} \times 10^6 \mu g/g \times 22.4\ \mu L/\mu mole \times 295°K/273°K}{MW(\mu g/\mu mole) \times \text{Flow(Lpm)} \times \text{exposure duration(min)}}$$

2.16. EXPERIMENTAL EVALUATIONS

2.16.1. VIABILITY CHECKS

Animals were observed, in their cages, for mortality and signs of severe toxic or pharmacologic effects twice daily.

2.16.2. PHYSICAL EXAMINATIONS

Exposure Observations

All visible animals were observed as a group at least once during each exposure.

Detailed Physical Examinations

Animals were removed from their cages and examined twice pretest and once weekly during the study period. Examinations included observations of general condition, skin and fur, eyes, nose, oral cavity, abdomen and

Detailed Physical Examinations

Animals were removed from their cages and examined twice pretest and once weekly during the study period. Examinations included observations of general condition, skin and fur, eyes, nose, oral cavity, abdomen and external genitalia as well as evaluations of respiration and palpation for tissue masses.

2.16.3. OPHTHALMOSCOPIC EXAMINATION

Method

Lids, lacrimal apparatus and conjunctiva were examined visually. The cornea, anterior chamber, lens, vitreous humor, retina and optic disc were examined by indirect ophthalmoscopy.

Mydriacyl 1% was used to induce mydriasis.

Examination Schedule

Pretest:	Test Day -5
Termination:	Test Day 91
Recovery:	Test Day 122

2.16.4. BODY WEIGHT

Animals were removed from their cages and weighed twice pretest, and once weekly during treatment and recovery. Terminal body weights for the rats were obtained after fasting just prior to necropsy. Terminal body weights for the mice were obtained just prior to necropsy and were not fasted.

2.16.5. FOOD CONSUMPTION

Rats

Feed was available without restriction (except during exposure) 7 days/week. Animals were presented with full feeders weighing 570 grams (includes weight of feed, jar and lid). After 6 days, feeders were reweighed and the resulting weight was subtracted from the full feeder weight to obtain the grams consumed per animal over the 6-day period. Food consumption was measured (weighed) weekly, beginning one week prior to treatment initiation.

Mice

Feed was available without restriction (except during exposure) 7 days/week. Animals were presented with full feeders weighing 190 grams (includes weight of feed, jar and lid). After 3 days, feeders were reweighed and the resulting weight was subtracted from the full feeder weight to obtain the grams consumed per animal over the 3-day period. Food consumption was measured (weighed) weekly, beginning one week prior to treatment initiation.

Calculation

$$\text{Food Consumption (g/kg/day)} = \frac{\text{grams of food consumed}}{\text{body weight (kg)}^{a}} \div \text{\# days}$$

Food Consumption (g/interval) = grams of food consumed

[a]The average of the current and previous weight was used for the rats. The current weight was used for the mice.

2.17. CLINICAL PATHOLOGY LABORATORY STUDIES

Rats

Blood for hematology and coagulation studies was obtained via the orbital sinus (retrobulbar venous plexus) under light CO_2/O_2 anesthesia. Blood for clinical chemistry studies was obtained via the abdominal aorta under CO_2/O_2 anesthesia. Animals were fasted overnight prior to blood collection.

Mice

Blood for hematology and clinical chemistry studies was obtained via the orbital sinus (retrobulbar venous plexus) under light CO_2/O_2 anesthesia.

2.17.1. HEMATOLOGY

Method of Blood Collection

Blood for hematology studies was collected into tubes containing EDTA anticoagulant. Blood for coagulation studies (rats only) was collected into tubes containing sodium citrate anticoagulant.

Collection Interval

Rats

Termination: Test Days 94 and 95

Mice
Termination: Test Day 93

Number of Animals Bled

10 animals/sex/group/species (as available)

Analysis of Blood Samples

Blood samples were analyzed as follows:

Technicon® H-1™ Hematology System, Bayer Corporation.

Hemoglobin concentration
Hematocrit
Erythrocyte count
Platelet count
Mean corpuscular volume
Mean corpuscular hemoglobin
Mean corpuscular hemoglobin concentration
Total leukocyte counts
Differential leukocyte count[1]
Absolute lymphocytes (*calculated value; total WBC x % lymphocyte value ÷ 100*)[1]
Absolute segmented neutrophils (*calculated value; total WBC x % segmented neutrophil ÷ 100*)[1]

Photo-optical clot detection system, COAG-A-MATE® X2, Organon Teknika Corp.

Prothrombin time
Activated partial thromboplastin time

Other

Reticulocyte count (Henry, 1991)

[1]When questionable values were obtained, manual differential leukocyte counts (Henry, 1991) and absolute value calculations were performed for verification.

2.17.2. CLINICAL CHEMISTRY

Method of Blood Collection

Blood for clinical chemistry studies was collected into tubes with no anticoagulant, allowed to clot, and centrifuged to obtain serum.

Collection Interval

Rats
Termination: Test Days 94 and 95

Mice
Termination: Test Day 93

Number of Animals Bled

10 animals/sex/group/species (as available)

Analysis of Blood Samples

Blood samples were analyzed as follows (when inadequate volume of serum was available, a priority list in the protocol was followed):

Hitachi 717, Boehringer Mannheim Corporation Automatic Analyzer.

Aspartate aminotransferase (*Kinetic - Modified IFCC Technique*)
Alanine aminotransferase (*Kinetic - Modified IFCC Technique*)
Alkaline phosphatase (*AMP Buffer - Modified Bessey-Lowry-Brock Technique*)
Lactate dehydrogenase (*Lactate-pyruvate Technique*)
Sorbitol dehydrogenase (*Sigma Diagnostics NADH*)
Blood urea nitrogen (*Modified Urease Technique*)
Creatinine (*Jaffe Reaction - Kinetic - Alkaline Picrate*)
Glucose (*Hexokinase Method*)
Creatine kinase (*Szasz-NAC Activated Method*)
Total protein (*Biuret Technique*)
Albumin (*Bromocresol Green Method*)
Globulin (*calculated value; total protein - albumin*)
Albumin/globulin ratio (*calculated value; albumin ÷ globulin*)
Total bilirubin (*Modified Jendrassik and Grof Method*)

Direct bilirubin conjugated (*Modified Jendrassik and Grof Method*)
Indirect bilirubin unconjugated (*calculated value; total bilirubin - direct bilirubin*)
Sodium (*Ion Selective Electrode*)
Potassium (*Ion Selective Electrode*)
Chloride (*Ion Selective Electrode*)
Calcium (*Cresolphthalein Complexone Method*)
Inorganic phosphorus (*Phosphomolybdate-UV Method*)
Gamma-glutamyl transferase (*Kinetic - Szasz, G.*)

2.17.3. URINALYSIS

Method of Urine Collection

For rats only, urine was collected in metabolism cages into iced (wet ice) containers. Urinalysis was performed on 16 hour collected samples from fasted animals.

Collection Intervals

Termination: Test Days 94 and 95
Recovery: Test Day 123

Number of Animals Sampled/Interval

10 rats/sex/group (as available)

Analysis of Urine Samples

The following parameters were analyzed using a freshly voided urine sample.

Appearance
Specific gravity (*Clinical Refractometer, Atago Uricon-N*)

A Clinitek 200+® Urine Chemistry Analyzer, Bayer Corporation, Diagnostics Division, was used for analyzing the following parameters.

Protein
Glucose
Ketones

Occult blood (semi-quantitatively)
pH
Bilirubin
Urobilinogen

Protein results of 100 mg/dL or greater were verified using a three percent sulfosalicylic acid test. Positive bilirubin results were confirmed via Ictotest® reagent tablets (Bayer Corporation, Diagnostics Division) (Henry, 1991).

Microscopic examination of sediment was performed on centrifuged urine samples via manual microscopy (Henry, 1991).

Osmolality (*Advanced Diagnostic Osmometer, Model 3DII, Advanced Instruments Inc.*)

Hitachi 717, Boehringer Mannheim Corporation Automatic Analyzer .

Creatinine (*Jaffe Reaction - Kinetic Method*)
N-acetyl-β-D-glucosamidase (*standard Boehringer Mannheim*)

2.18. POSTMORTEM

2.18.1. NECROPSY INFORMATION

Method of Euthanasia

Rats
For the Terminal Sacrifice, animals were exsanguinated following carbon dioxide/oxygen inhalation. For the Recovery sacrifice, animals were exsanguinated following carbon dioxide inhalation.

Mice
For the Terminal and Recovery sacrifices, animals were exsanguinated following carbon dioxide inhalation.

Necropsy Intervals

Rats
Termination: Test Days 94 and 95
Recovery: Test Day 123

Mice
Termination: Test Day 93
Recovery: Test Day 122

Number of Animals/Interval

10 animals/sex/group/species (as available)

Necropsy Order

A necropsy schedule was established to ensure that approximately equal numbers of males and females were examined on each day of necropsy and that examination of animals of both sexes were performed at similar times of the day throughout the necropsy period. In addition, rats and mice were sacrificed in the following order: one animal each was selected from the control, high-, mid-, and low-exposure groups and sacrificed in that order. This sequence was repeated until all the animals were sacrificed.

2.18.2. MACROSCOPIC EXAMINATIONS

Complete macroscopic postmortem examinations were performed on all animals killed at a scheduled sacrifice interval immediately after death. The macroscopic postmortem examination included examination of the external surface and all orifices; the external surfaces of the brain and spinal cord; the organs and tissues of the cranial, thoracic, abdominal and pelvic cavities and neck; and the remainder of the carcass for the presence of macroscopic morphologic abnormalities. The rats were fasted prior to scheduled sacrifices. The mice were not fasted prior to scheduled sacrifice.

Animals which were found dead or which were killed accidentally were similarly examined as soon as it was practically possible.

2.18.3. ORGAN WEIGHTS

Organs indicated in Table I (below) were weighed for all animals at the scheduled sacrifice intervals. Prior to weighing, the organs were carefully dissected and properly trimmed to remove adipose and other contiguous tissues in a uniform manner. Organs were weighed as soon as possible after dissection in order to avoid drying. Paired organs were weighed together.

2.18.4. TISSUES PRESERVED AND EXAMINED HISTOPATHOLOGICALLY

The tissues listed in table below were obtained at the scheduled sacrifice intervals and preserved for all animals. See the Pathology Reports located in Appendices N and Z for the rats and mice, respectively, for materials and methods of histopathology processing and microscopic examinations as well as a list of tissues examined microscopically.

ORGAN NAME	NO.[a]	WEIGHED	PRESERVED
adrenal glands	2	X	X
aorta (thoracic)	1		X
bone (sternum, femur with articular surface)	2		X
bone marrow (sternum, femur)[b]	2		X
brain (medulla/pons, cerebrum and cerebellum)	3	X	X
esophagus	1		X
eyes with optic nerve	4		X
heart	1		X
kidneys	2	X	X
lacrimal glands	2		X
large intestine (cecum, colon, rectum)	3		X
larynx	4		X
liver	2	X	X
lungs (with mainstem bronchi)	5	X	X
lymph node (mesenteric)	1		X
nasopharyngeal tissues (including nasal turbinates)	4		X

ORGAN NAME	NO.[a]	WEIGHED	PRESERVED
mammary gland (inguinal)	1		X
nerve (sciatic)	1		X
ovaries	2	X	X
pancreas	1		X
pituitary gland	1		X
prostate gland	1	X	X
salivary glands (submandibular)	2		X
seminal vesicles	2	X	X
skeletal muscle (*Biceps femoris*)	1		X
skin	1		X
small intestine (duodenum, ileum, jejunum)	3		X
spinal cord (cervical, thoracic, lumbar)	3		X
spleen	1		X
stomach	1		X
testes with epididymides	2	X	X
thymic region	1		X
thyroid/parathyroid glands	4		X
trachea	1		X
urinary bladder	1	X	X
uterus (body/horns) with cervix	3	X	X
vagina	1		X
Zymbal's gland	2		X
tissues with macroscopic findings including tissue masses			X

[a]Number of organs/sections preserved/examined.

[b]Qualitative examination (no differential count).

Preservatives

Eyes - glutaraldehyde/paraformaldehyde.
All other tissues - 10% neutral buffered formalin.

Lungs and urinary bladder were infused with formalin prior to their immersion into a larger volume of the same fixative. The nasopharyngeal tissues were flushed with formalin prior to their immersion into a larger volume of the same fixative.

2.19. STATISTICAL ANALYSIS

The following methods were used to statistically analyze the data for the specified parameters. For each method, mean values of all exposure groups were compared to the mean value for the control group at each time interval with the following exceptions for Methods 1 and 2:

Statistical evaluations were not performed when the standard deviation for the control group was 0.

Exposure groups were eliminated from statistical analysis if their standard deviation was 0.

2.19.1. METHOD 1

Parameters

body weight
change from base body weight
food consumption (g/kg of body weight/day)

Method of Analyses

Body weight and food consumption data was initially analyzed by Bartlett's test (Snedecor and Cochran, 1989) for equal variance. If the results of the test were significant at the less than 0.01 level, the data were transformed by Blom's Normalized Rank Test (Blom, 1958) before continuing with any further analysis. The data were then analyzed by a standard two-way analysis of variance, ANOVA (Snedecor and Cochran, 1989). The residuals from this analysis were then tested for normality using the Shapiro-Wilk's test.

If the residual data were normally distributed and the ANOVA analysis was significant at the 0.05 level, the data were then analyzed using a Dunnett's test (Dunnett, 1964) followed by analysis using standard tests for linear regression and lack of fit (Snedecor and Cochran, 1989). If the results of the ANOVA were not significant at the 0.05 level, then the data were just analyzed using standard tests for linear regression and lack of fit (Snedecor and Cochran, 1989).

If the residual data were not normally distributed at the 0.01 level, the data were transformed using Blom's Normalized Rank Test and reanalyzed using ANOVA and regression analysis. If the residuals of the transformed, reanalyzed data were normal, the data were then analyzed using Dunnett's test followed by analysis for linear regression and lack of fit. If the residuals of the transformed, reanalyzed data were not normal, the resulted were noted as suspect and the data were analyzed using Dunnett's test followed by analysis for linear regression and lack of fit.

2.19.2. METHOD 2

Parameters

food consumption (g/interval)
hematology
clinical chemistry
organ weights
urine pH
urine specific gravity
urine osmolality
N-acetyl-β-D-glucosamidase
urine creatinine

Method for Multiple Group Analyses

Employed when more than one treated group is compared to control.

Statistical evaluation of equality of means was made by the appropriate one way analysis of variance technique, followed by a multiple comparison procedure if needed. First, Bartlett's test (Snedecor and Cochran, 1967) was performed to determine if groups had equal variance. If the variances were equal, parametric procedures were used; if not, nonparametric procedures were used. The parametric procedures were the standard one way ANOVA (Snedecor and Cochran, 1967) using the F distribution to assess significance. If significant differences among the means were indicated, Dunnett's test (Dunnett, 1955, 1964) was used to determine which means were significantly different from the control. If a nonparametric procedure for testing equality of means was needed, the Kruskal-Wallis test (Hollander and Wolfe, 1973) was used, and if differences were indicated Dunn's summed rank test (Hollander and Wolfe, 1973) was used to determine which treatments differed from control.

A statistical test for trend in the exposure levels was also performed. In the parametric case (i.e., equal variance) standard regression techniques with a test for trend and lack of fit were used (Snedecor, and Cochran, 1967). In the nonparametric case Jonckheere's test (Hollander and Wolfe, 1973) for monotonic trend was used.

The test for equal variance (Bartlett's) was conducted at the 1%, two-sided risk level. All other statistical tests were conducted at the 5% and 1%, two-sided risk level.

Method for Two Group Analyses

Employed when only one treated group is compared to control.

The variances of the two groups were tested for equality using the F test (Gill, 1978). If the variances were equal, a standard independent two sample t-test (Gill, 1978) was used to determine equality of means. If the variances differed at the 1% level of significance, Welch's t-test (Gill, 1978) was used to determine equality of means. t-tests were conducted at the 5% and 1% two-sided risk level.

2.20. DATA STORAGE

All raw data, preserved specimens, and retained samples, as well as the original study protocol and the original final report are to be maintained in the Archives of the Testing Facility upon completion of the study for a period of one year, after which the Sponsor will archive the previously mentioned materials.

2.21. PROTOCOL DEVIATIONS

The following protocol deviations occurred during the study but were not considered to have compromised the validity or integrity of the study:

1. The body weight range at initiation of exposures for the male rats was 198 to 233 grams; however, the protocol required that the range be 160 to 210 grams at initiation of exposures.

2. On 1 April 1998, the temperature and humidity in the Group IVB chamber was measured and recorded 3 times during the exposure due to the dislocation of the gauge within the chamber; however, the protocol required that the temperature and humidity be measured and recorded 13 times/chamber/day.

3. Animal number 4650 was sacrificed during the recovery period and had a microscopic examination of all tissues required for animals sacrificed during the termination period; however, the protocol required that only selected tissues be examined microscopically for animals sacrificed during the recovery period.

4. A detailed physical observation was not recorded for animal number 4651 during Week 11; however, the protocol required that observations be performed weekly.

5. Ophthalmoscopic examinations were performed on all surviving test animals at the end of the recovery period; however, the protocol only required examinations pretest and at study termination.

6. The tissue, blocks, slides as well as the final pathology report are retained in the archives of the Testing Facility; however, the protocol required that these items be retained at Huntingdon Life Sciences, Eye Research Centre.

3. RESULTS AND DISCUSSION

3.1. CHAMBER MONITORING

(Appendix A)

Prestudy chamber distribution analyses showed the test material was evenly distributed within each chamber.

The target and mean (± standard deviation) analytical (measured by infrared spectrophotometry) and nominal concentrations are summarized as follows:

Group	Target Concentration (ppm)	Analytical Concentration (ppm)	Nominal Concentration (ppm)
IA (mice)	0	0.00±0.0	-
IB (rats)	0	0.00±0.0	-
II	20	19.9±1.0	19±0.7
III	80	78.9±4.6	83±3.3
IVA (mice)	200	196.3±8.3	226±6.1
IVB (rats)	400	395.5±18	395±15

The variation (or difference) between the mean exposure concentration and the respective target concentration was considered acceptable. The differences between measured and nominal concentrations were typical for this type of exposure and considered reasonable within the limits of experimental precision.

Chamber environmental conditions averaged 25°C and 47% relative humidity.

Particle size distribution measurements for the exposures are summarized as follows:

Group	Mass Median Aerodynamic Diameter (µm)	Geometric Standard Deviation	Total Mass Concentration (µg/m^3)
IA (mice)	2.774	2.249	3.47
IB (rats)	2.949	2.296	4.66
II	3.628	2.260	3.30
III	4.216	2.288	3.20
IVA (mice)	3.793	2.324	3.15
IVB (rats)	4.872	2.138	1.52

These results indicated that all test material exposures were vapor only, as expected, since their measurements were comparable to air control measurements.

3.2. MORTALITY

(Rats: Appendix B; Mice: Appendix O)

No mortality occurred in any of the rats. Three female mice from Group IV were found dead on Test Days 39, 56 and 74. The cause of death was not established by in-life or post-life examinations but these deaths were considered treatment-related. Additional deaths in one mouse from each VCMX exposure group were attributed to accidental cause.

3.3. PHYSICAL OBSERVATIONS

3.3.1. IN-CHAMBER OBSERVATIONS

(Table 1)

The test animals were generally unremarkable during the exposure periods throughout the study. Sporadic instances of eye closure, excessive lacrimation, mucoid nasal discharge, excessive salivation, poor condition, labored breathing and hind limb impairment were noted in the Group IV animals but these were not seen in any consistent exposure-level-related or species-related pattern.

3.3.2. WEEKLY DETAILED OBSERVATIONS

(Rats: Table 2; Appendix C)

Test material exposed rats of both sexes in Group IV showed increased incidences of facial stains (red/black/brown) throughout the exposure period with a decrease in the recovery period. A transient increase in the incidence of nasal discharge was seen in both sexes of this group during the first 4 weeks of treatment. A transient increase in the incidence of yellow ano-genital staining was also seen in both sexes of this group during the last few weeks of treatment.

(Mice: Table 12; Appendix P)

Test material exposed male mice in Group IV showed an increased incidence of yellow ano-genital staining during the exposure and recovery periods.

3.4. OPHTHALMOSCOPIC EXAMINATIONS

(Rats: Table 3 & Appendix D; Mice: Table 13 & Appendix Q)

No effect of the test material was evident from ophthalmoscopic examinations. Observations for test material exposed rats and mice were comparable to those for air control animals.

3.5. BODY WEIGHTS

(Rats: Figures 2-3, Table 4-5 & Appendices E-F)

Group IV male rats showed lower absolute body weights and decreased body weight gains throughout the exposure period with recovery after termination of exposures.

(Mice: Figures 8-9, Tables 14-15 & Appendices R-S)

Group IV male mice showed lower absolute body weights and decreased body weight gains during most of the exposure period with a recovery of weight gain after termination of exposures.

No effects on body weight gains were seen in females of either species.

3.6. FOOD CONSUMPTION

(Rats: Figures 4-7, Table 6-7 & Appendices G-H)

Test material exposed rats showed decreased and/or increased food consumption (g/kg/day) in Group IV males throughout the study. These varying differences were not considered evidence of a test material effect.

(Mice: Figures 10-13, Tables 16-17 & Appendices T-U)

Values for test material exposed mice were generally comparable to those for air control mice. Statistically significant decreases in food consumption (calculated as g/kg/day and g/interval) were seen in Groups II and III animals (both sexes) frequently during the first 6 weeks of exposure. Occasional differences in Group IV were seen also. However, the pattern of these differences was not consistent with exposure-related changes and there were no correlating body weight changes. Thus, these feed consumption differences were not considered related to toxicity of the test material.

3.7. CLINICAL LABORATORY STUDIES

3.7.1. HEMATOLOGY

(Rats: Table 8 & Appendices I & J; Mice: Table 18 & Appendices V & W)

Values for test material exposed rats and mice were comparable to values for the air control animals at the Terminal Sacrifice. Therefore, hematology parameters were not evaluated at the Recovery Sacrifice.

3.7.2. CLINICAL CHEMISTRY

(Rats: Table 9 & Appendix K; Mice: Table 19 & Appendix X)

Values for test material exposed rats and mice were comparable to those for the air control animals at the Terminal Sacrifice or exhibited normal variability. Therefore, clinical chemistry parameters were not evaluated at the Recovery Sacrifice.

3.7.3. URINALYSIS

(Rats: Table 10 & Appendix L)

At the Terminal Sacrifice, urinalysis values for test material exposed rats were comparable to the air control animals except that the Group IV males and females had significantly lower pH values and slight increases in ketones, bilirubin and urobilinogen. At the Recovery Sacrifice, these differences were no longer evident except ketones were still somewhat increased in Group IV males.

Urinalysis was not performed for the mice, as per protocol.

3.8. ORGAN WEIGHTS

(Rats: Table 11 & Appendix M)

No direct effect of VCMX exposure was evident on organ weights of rats. Increased relative weights for several organs in high-exposure group males were consistent with low body weights in this group. A slight increase in absolute and relative liver weights was observed in females from the Group IV recovery. In the absence of similar findings in the males or in either sex at the Terminal Sacrifice or any corresponding histopathology findings (see next section), these differences were not considered treatment-related. Organ weights for other test material exposed groups, and Group IV recovery males, were also generally comparable to control values, exhibited normal variability and/or lacked an exposure-level-related response.

(Mice: Table 20 & Appendix Y)

Ovary weights for mice exposed to the highest concentration were statistically significantly decreased, relative to control values, at both Terminal and Recovery Sacrifice intervals. This is consistent with microscopic pathology observations of absence of ovarian follicles in these animals. Other organ weights for mice were generally comparable between control and test material exposed groups, exhibited normal variability and/or lacked an exposure-level-related response. Therefore, no other effects of VCMX were evident.

3.9. PATHOLOGY

3.9.1. MACROSCOPIC

(Rats: Appendix N)

There were no findings in the rats considered to be related to treatment with the test compound. There was a very low level of findings which might be expected in rats of this age.

(Mice: Appendix Z)

There were a number of mice in all exposure groups including control with fluid filled lungs. Fluid was also seen in the trachea of these animals. These findings in the mice were probably an artifact of the CO_2 anesthesia used for euthanasia. All other findings were of the types that might be expected in mice of this age and occurred at a very low frequency.

3.9.2. MICROSCOPIC

(Rats: Appendix N)

At the end of the 13-week exposure and 4-week post-exposure recovery periods, findings related to the test material were seen in the nasoturbinal tissues of the rats.

At the end of 13-Weeks of exposure, thin sensory epithelium (slight to moderate) covering the nasoturbinal tissues was the only test material related microscopic finding. This occurred with comparable severity in 3/10 males and 7/10 females in the 400 ppm group and in 3/10 females in the 80 ppm group. A similar finding, of slight severity, in 1/10 females in the Air Control group was considered to be incidental.

At the end of the 4-week post-exposure recovery period, thin sensory epithelium (minimal to slight) persisted in 6/10 males and 4/10 females in the 400 ppm group indicating that recovery had not occurred. A similar finding, of slight severity, in 2/10 males in the Air Control group was considered to be incidental.

Thinning of the sensory epithelium of the nasoturbinal tissues was considered to be consistent with a localized effect of the test material. This is supported by the fact that there were no test material related findings in the lungs nor in other tissues and organs which would be indicative of a systemic effect.

At the end of both the exposure and post-exposure recovery periods, other microscopic findings in the nasoturbinal tissues occurred with comparable incidence and severity in rats from the Air Control and test material exposure groups or they occurred sporadically. None of the findings had a sex/exposure level relationship. These incidental findings were not considered to be related to the whole body exposure to the test material. Microscopic findings in other tissues and organs occurred with comparable incidence and severity or they occurred sporadically in rats in the Air Control group and in the groups exposed to the test material. These incidental findings, not considered to be related the test material, have been seen in rats of this strain and age used in comparable studies conducted in this facility.

(Mice: Appendix Z)

At the end of the 13-week exposure and 4-week post-exposure recovery periods, microscopic findings related to the test material were seen in the ovaries and nasoturbinal tissues of the mice.

Ovaries

At the end of the 13-week exposure period, the number of ovarian follicles was reduced in 7/10 females in the 200 ppm group only. At the end of the 4-week recovery period, the number of ovarian follicles was still reduced in 6/10 females from the 200 ppm group indicating that recovery had not occurred. This effect on the ovaries was considered to be a direct systemic effect related to the exposure to the test material.

Nasoturbinal tissues

At the end of 13-weeks of exposure, findings related to the exposure to the test material, overall, occurred more frequently in females than in males. These were considered to be consistent with a direct localized effect of the test material. This is supported by the absence of test material related findings in the lungs and other tissues of the respiratory tract.

In the olfactory mucosa, the sensory epithelium was thin (minimal to moderate) in 1/10 males each in the 80 and 200 ppm groups and in 1/10 females each in the 20 and 80 ppm groups and 4/10 females in the 200 ppm group. In the affected mice in the 200 ppm group, the severity was moderate and the thinning of sensory epithelium was accompanied by slight to moderate loss of nerve fibers. Also, only in the males, there was slight necrosis of sensory epithelium in 1/10 in the 80 ppm group and in 2/10 in the 200 ppm group.

In the respiratory mucosa, loss of epithelial cilia (minimal to moderate) occurred in 1/10 males and 6/10 females in the 200 ppm group; overall severity was greatest in the females. A similar finding of slight severity in 1/10 females from the Air Control group was considered to be incidental. Focal thinning (slight) of the respiratory epithelium occurred in 1/10 males and 1/10 females in the 200 ppm group. Eosinophilia with blebbing (minimal to marked) of the respiratory epithelium occurred only in mice exposed to the test material (4/10 males each from the 80 and 200 ppm groups and 3/10, 7/10 and 2/10 females in the 20, 80 and 200 ppm groups respectively). Intracytoplasmic eosinophilic material similar to that seen in this study has been seen in mice in air control groups and in groups exposed to various test materials from other inhalation studies conducted in this facility. The exact nature of this material, presumed to be secretory, and reasons for its increase following exposure to a variety of test materials are uncertain but probably represents a nonspecific response to the inhalation of the test material.

At the end of the 4-week post-exposure recovery period, the sensory epithelium in mice in the 200 ppm group was thin in 1/10 males (slight) and 3/10 females (slight to moderate); slight necrosis in 2/10 females was accompanied by moderate loss of nerve fibers in 1/10 females. In the respiratory mucosa, loss of epithelial cilia (slight) occurred in 1/10 females in the 200 ppm group. Eosinophilia with blebbing (minimal to marked) of the respiratory epithelium occurred in 1/10 males and 3/10 females in the 200 ppm group and in 1/10 males from the Air Control group. In the nasoturbinal tissues, a comparison of incidence and severity of the test material related findings at the end of the exposure and recovery periods indicated that recovery had not occurred.

At the end of both the exposure and post-exposure recovery periods, microscopic findings in other tissues and organs occurred with comparable incidence and severity or they occurred sporadically in mice in the Air Control group and in the groups exposed to the test material. These incidental findings, not considered to be related to the test material, have been seen in mice of this strain and age used in comparable studies conducted in this facility.

An expert review of the changes in nasal epithelium from this study and the preliminary study was conducted to better characterize the lesions in relation to typical irritant materials. This report is included in Appendix EE.

4. CONCLUSION

Inhalation exposure of rats and mice to VCMX at concentrations of 20 ppm and higher, for 13 weeks, resulted in an exposure-level-related increase in nasal epithelium changes in both species, which were not completely reversible in high-concentration animals (mice, 200 ppm; rats, 400 ppm) held for a 4-week recovery period. These nasal effects were seen without any corresponding lung changes indicating a localized effect of test material exposure within the respiratory tract. Based on these findings, a NOEL for inhalation exposure to Vinyl Cyclohexene Monoxide (VCMX) was not established in this study. Systemic effects were limited to mortality and non-reversible ovarian degeneration in female mice exposed to the 200 ppm concentration, decreased body weight gains in male rats and mice, decreased urine pH and other minor urinary changes at the highest concentration (400 and 200 ppm, respectively).

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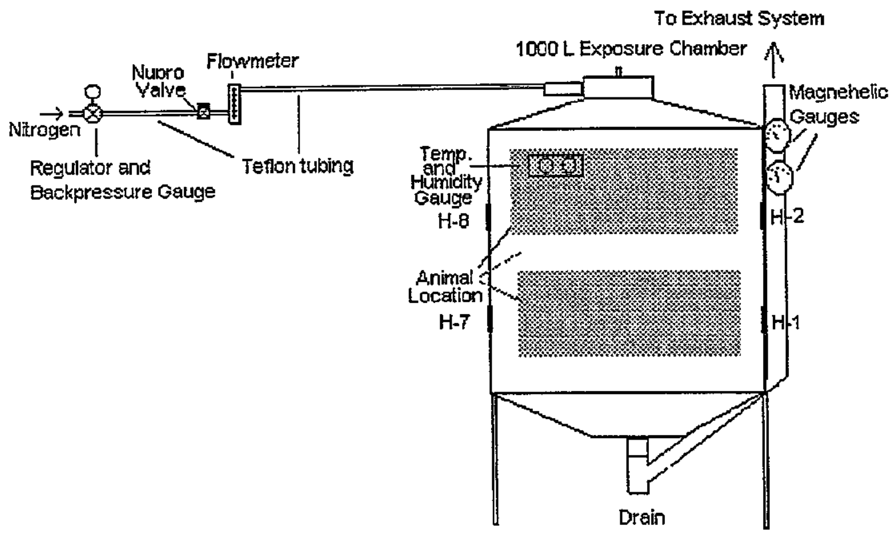
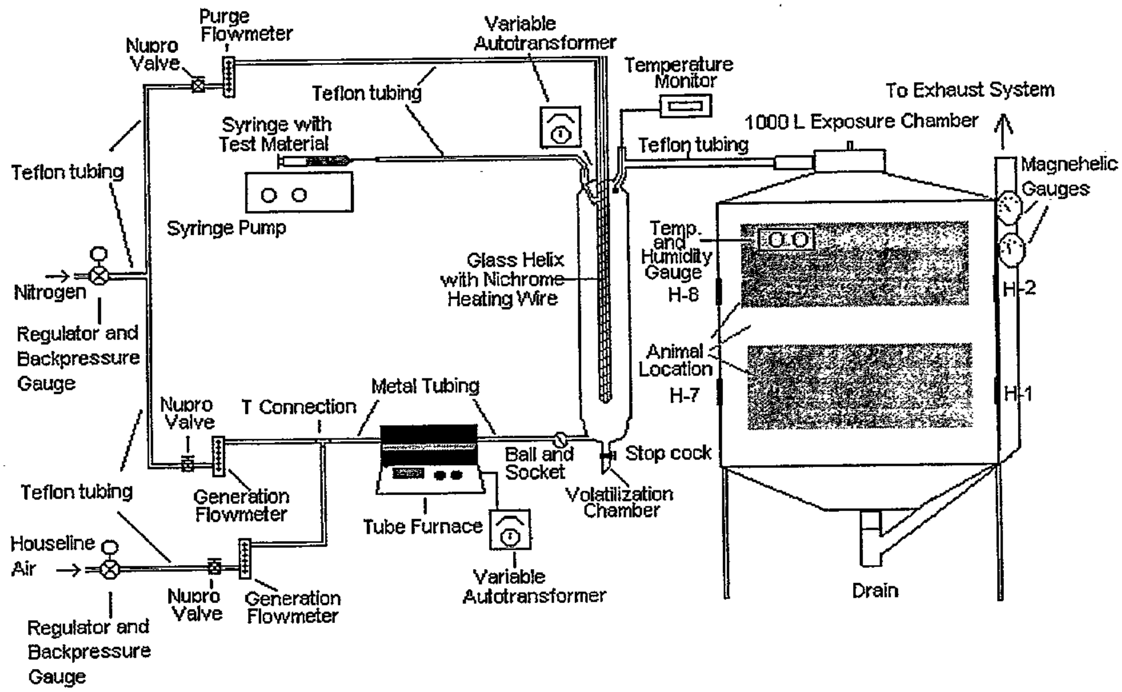
	Chamber Generation Systems and Whole-Body Exposure Chambers Groups IA and IB	Figure 1a



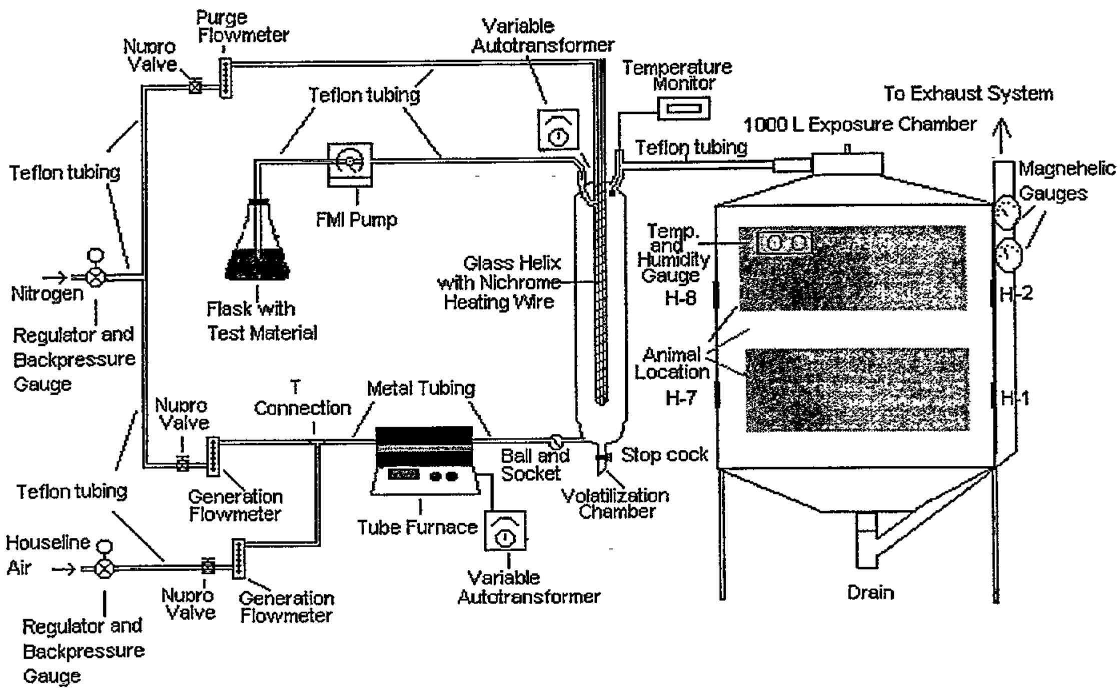
Note: Sampling Ports H-11 and H-13 were located on the back of the chamber and were used for prestudy distribution samples (see Appendix A).

	Chamber Generation Systems and Whole-Body Exposure Chambers Groups II and III	Figure 1b



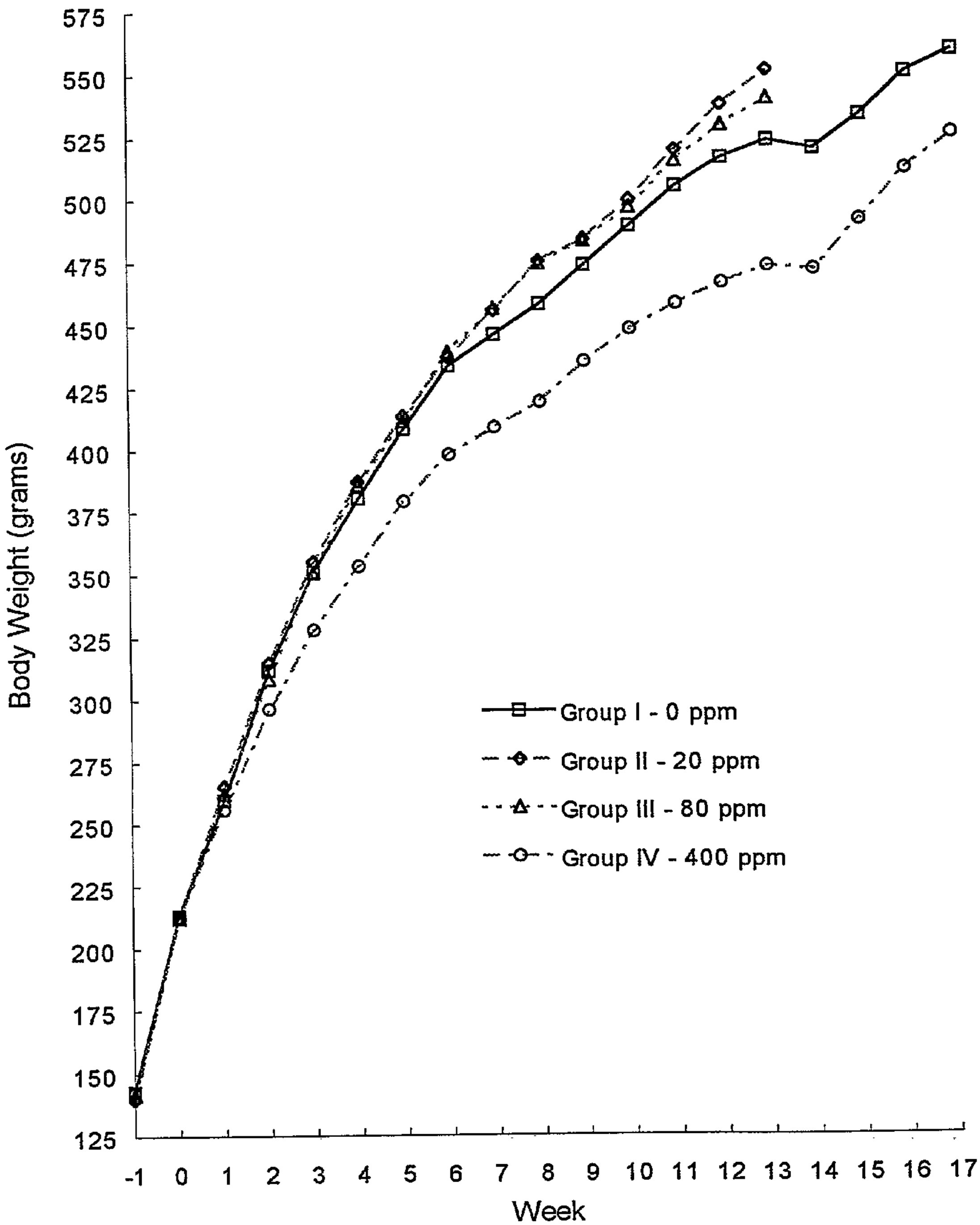
Notes: Sampling Ports H-11 and H-13 were located on the back of the chamber and were used for prestudy distribution samples (see Appendix A).

	Chamber Generation Systems and Whole-Body Exposure Chambers Groups IVA and IVB	Figure 1c

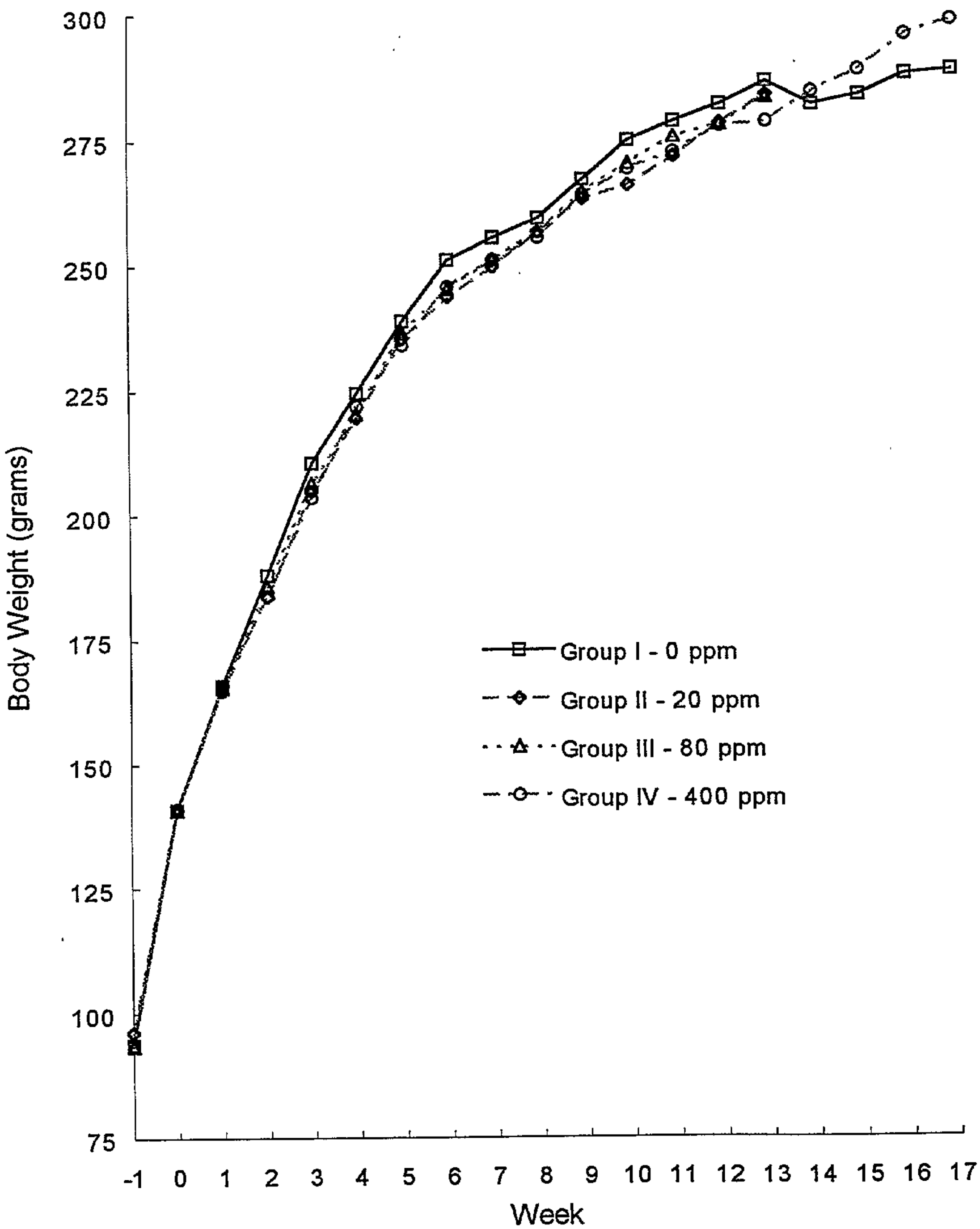


Notes: Sampling Ports H-11 and H-13 were located on the back of the chamber and were used for prestudy distribution samples (see Appendix A).

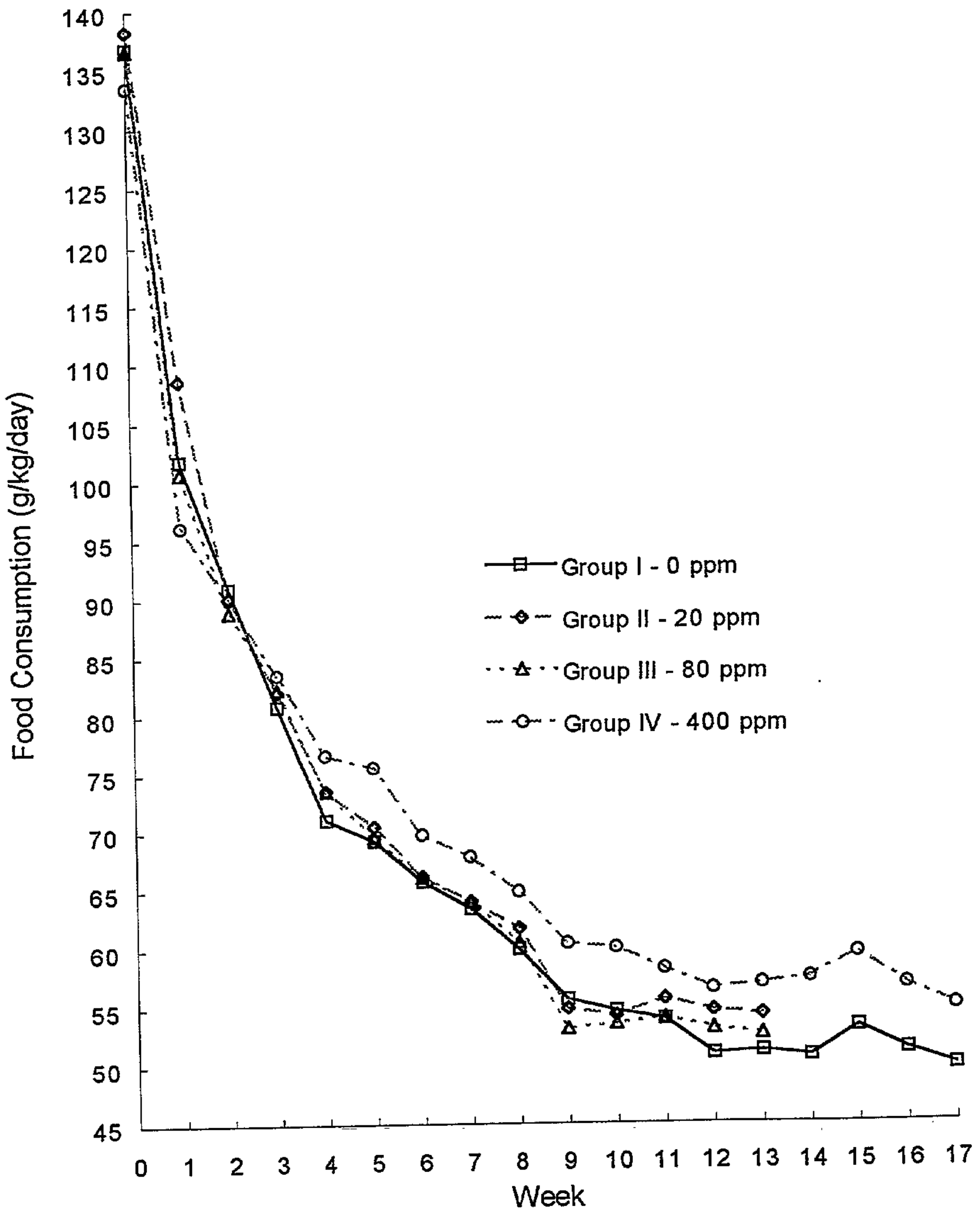
Males	Mean Body Weights Rats	Figure 2



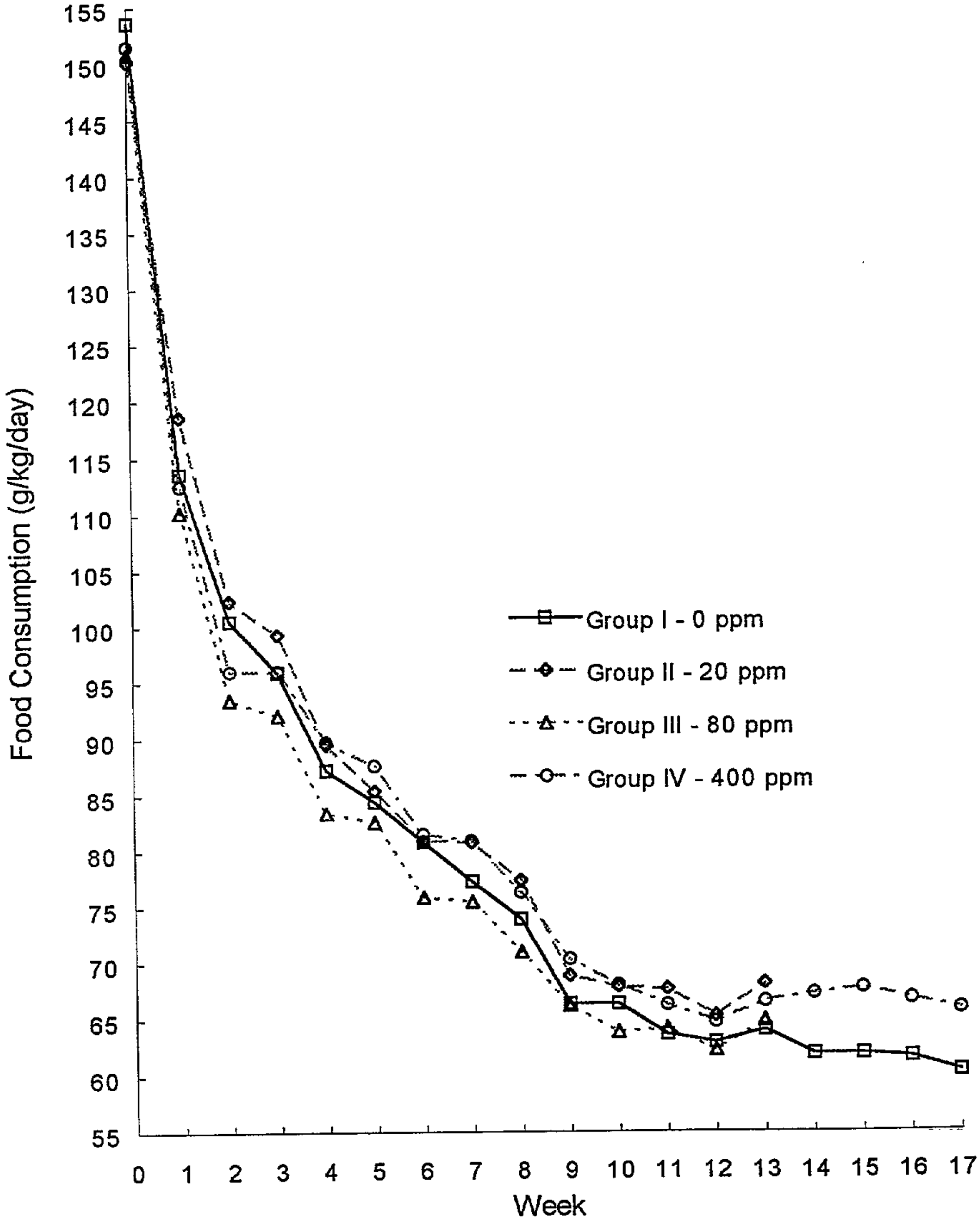
Females	Mean Body Weights Rats	Figure 3



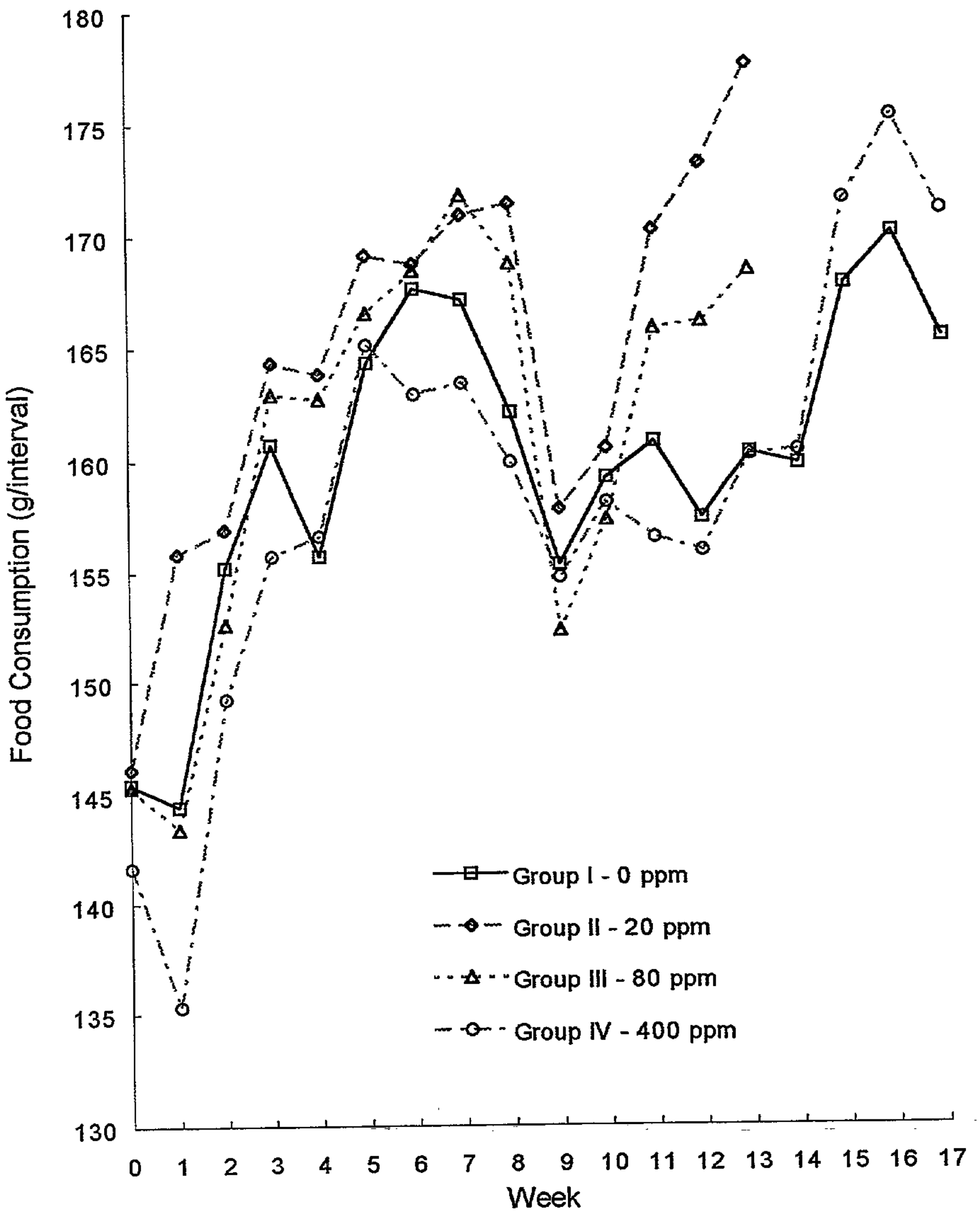
Males	Mean Food Consumption (g/kg/day) Rats	Figure 4



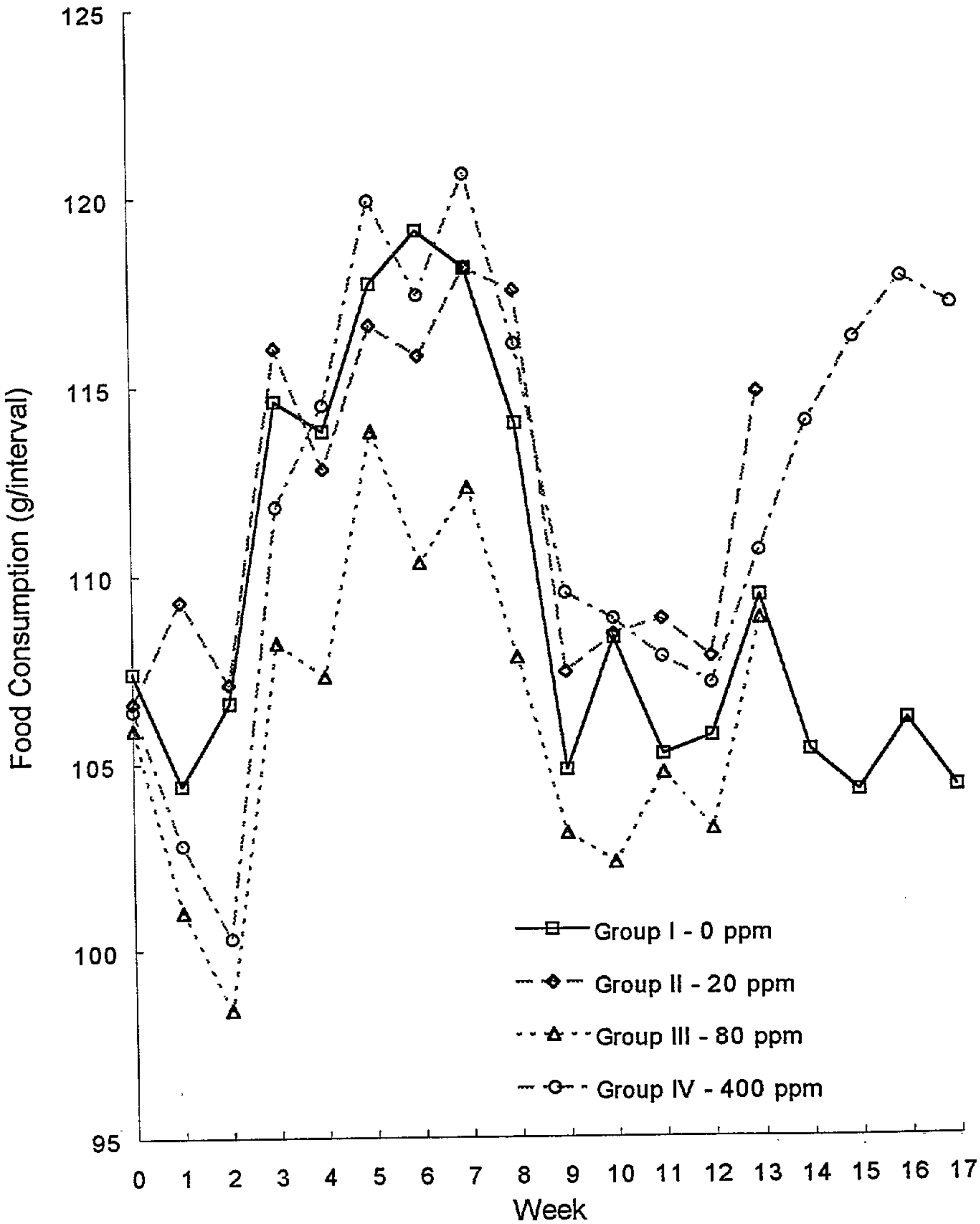
Females	Mean Food Consumption (g/kg/day) Rats	Figure 5



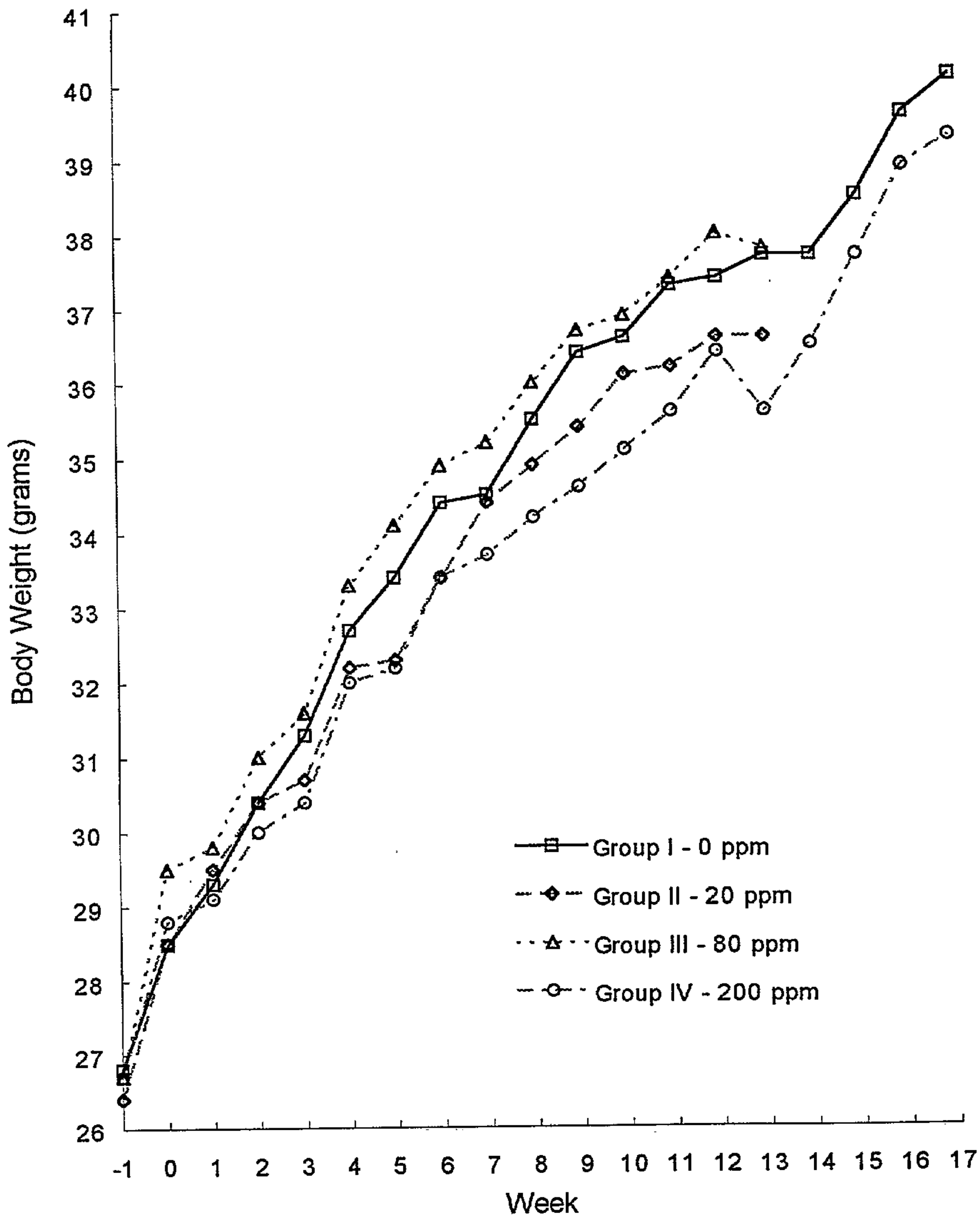
Males	Mean Food Consumption (g/interval) Rats	Figure 6



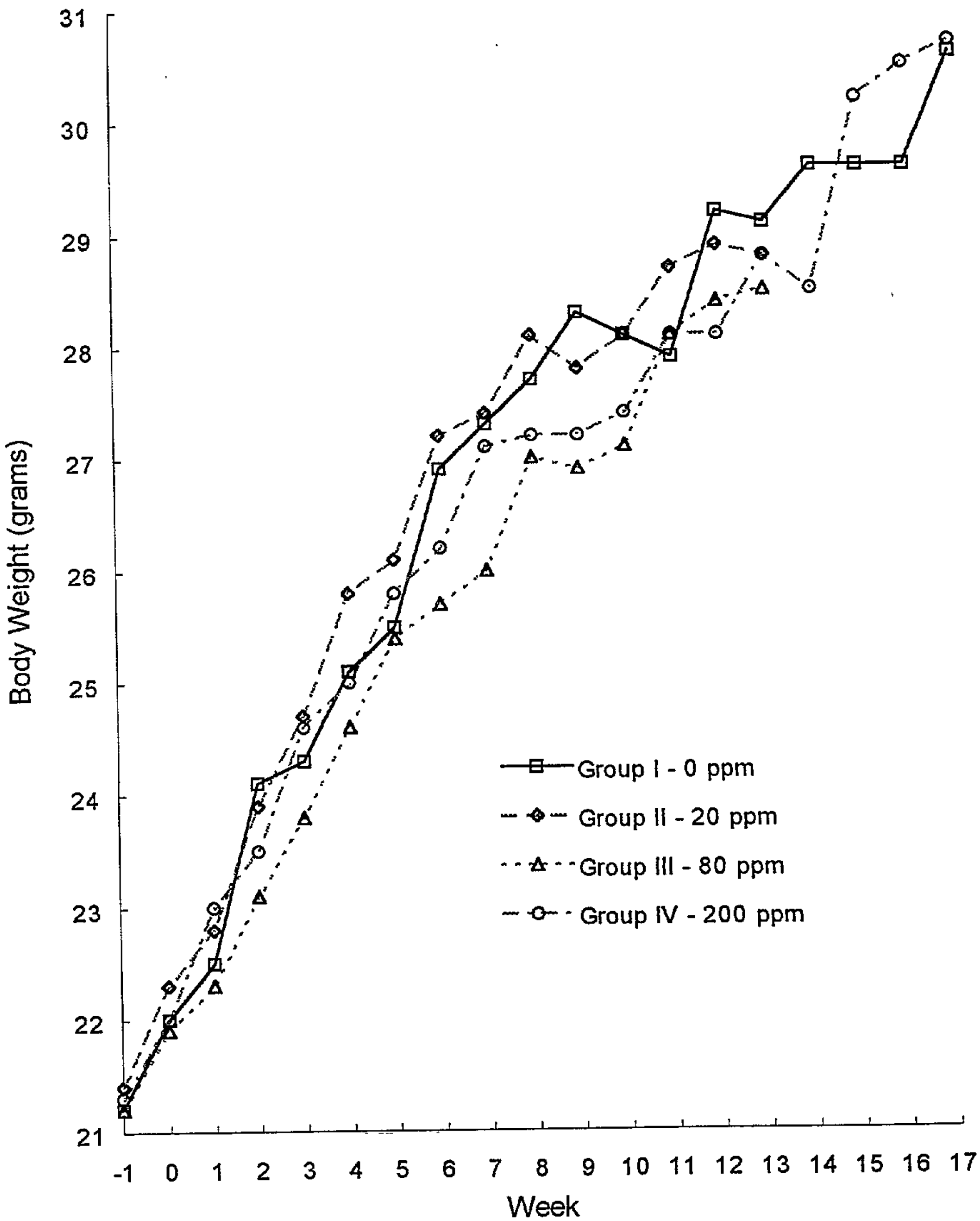
Females	Mean Food Consumption (g/interval) Rats	Figure 7



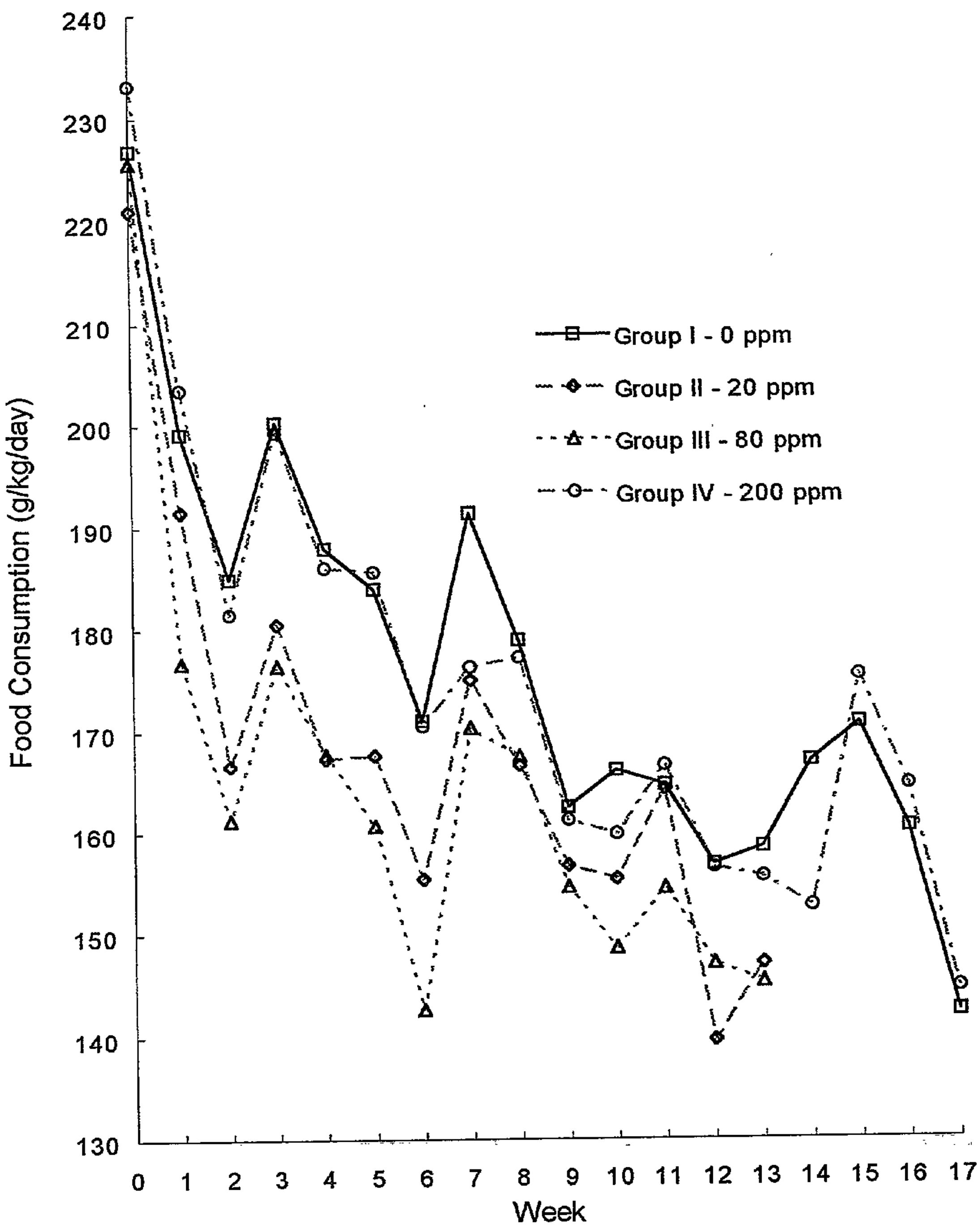
Males	Mean Body Weights Mice	Figure 8



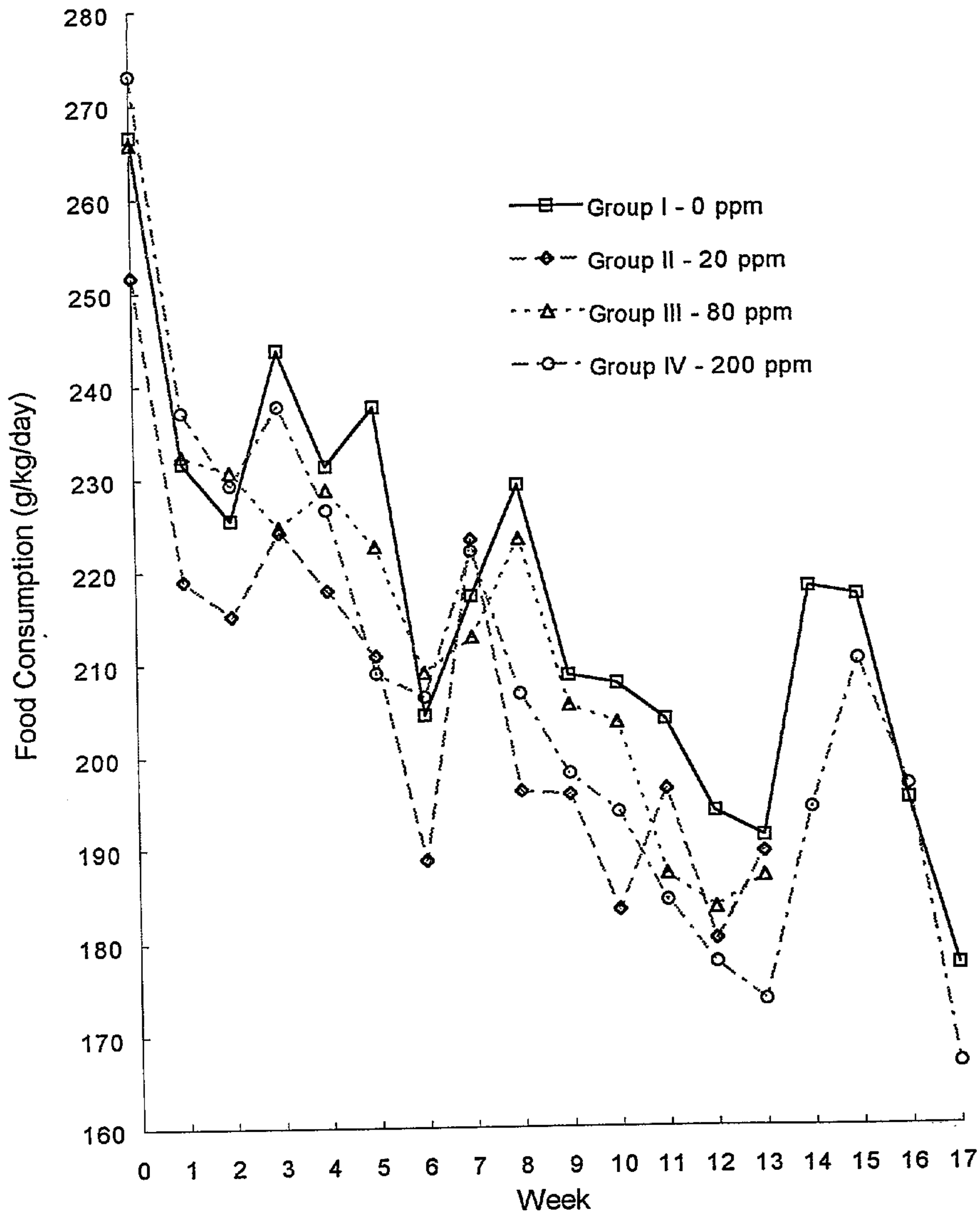
Females	Mean Body Weights Mice	Figure 9



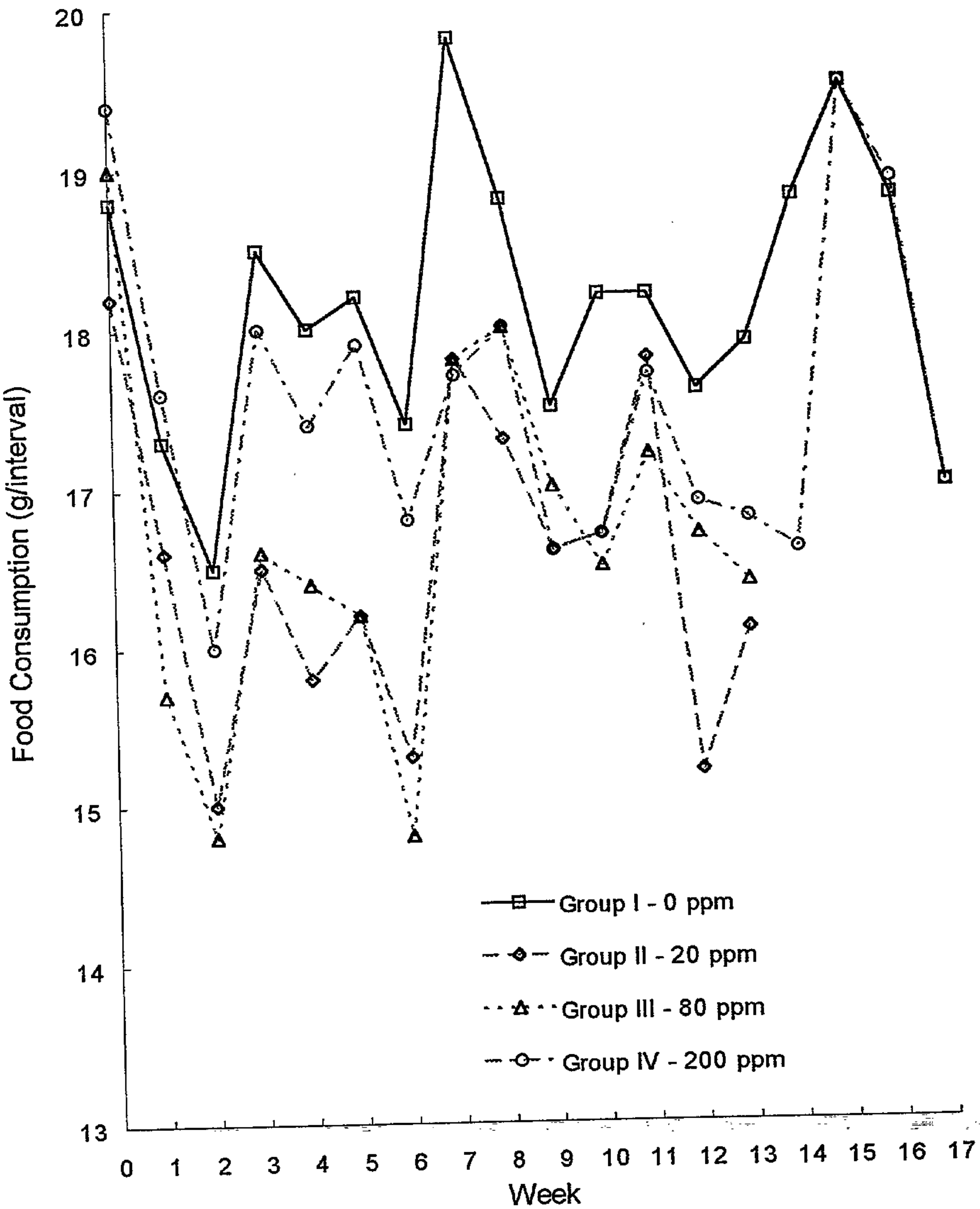
Males	Mean Food Consumption (g/kg/day) Mice	Figure 10



Females	Mean Food Consumption (g/kg/day) Mice	Figure 11



Males	Mean Food Consumption (g/interval) Mice	Figure 12



Females	Mean Food Consumption (g/interval) Mice	Figure 13